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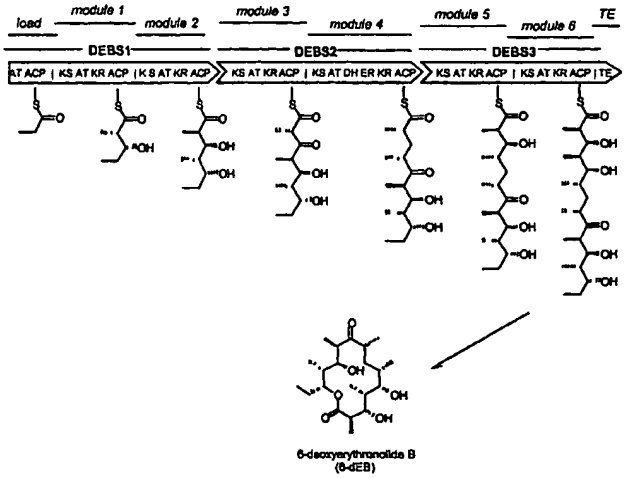
(54) Title: BIO-INTERMEDIATES FOR USE IN THE CHEMICAL SYNTHESIS OF POLYKETIDES



(57) Abstract: The present invention relates to compounds made by a subset of modules from one or more polyketide synthase ("PKS") genes that are used as starting material in the chemical synthesis of novel molecules, particularly naturally occurring polyketides or derivatives thereof. The biologically derived intermediates ("bio-intermediates") generally represent particularly difficult compounds to synthesize using traditional chemical approaches due to one or more stereocenters. In one aspect of the invention, an intermediate in the synthesis of epothilone is provided that feeds into the synthetic protocol of Danishefsky and co-workers. In another aspect of the invention, intermediates in the synthesis of discodermolide are provided that feed into the synthetic protocol of Smith and co-workers. By taking advantage of the inherent stereochemical specifity of biological processes, the syntheses of key intermediates and thus the overall syntheses of compounds like epothilone and discodermolide are greatly simplified.

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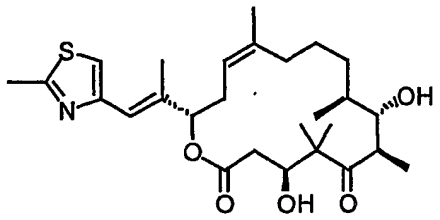
For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

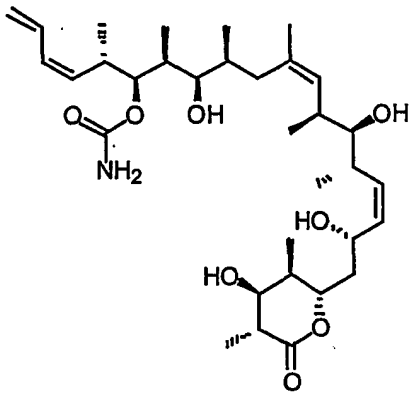
BIO-INTERMEDIATES FOR USE IN THE CHEMICAL SYNTHESIS OF POLYKETIDES

BACKGROUND

Produced naturally in many type of organisms including fungi and mycelial bacteria (particularly actinomycetes), polyketides are a structurally diverse class of compounds that are the source of many biologically active molecules. Two examples of polyketides that are of particular recent interest include epothilone (particularly epothilone D) and discodermolide.



epothilone D



(+)-discodermolide

Initial studies of these compounds in tubulin polymerization assays suggest they may act as potent anti-cancer agents. However, more extensive on going clinical investigations are hampered by the small quantities of epothilone and discodermolide that can be obtained from naturally occurring sources.

Several research groups have succeeded in the *de novo* chemical syntheses of epothilone and discodermolide. Syntheses of epothilones are described in, for example, Danishefsky *et al.*, "Synthesis of epothilones, intermediates thereto and analogues thereof," U.S. Patent Nos. 6,204,388 and 6,242,469 (both of which are incorporated herein by reference). Syntheses of discodermolide are described in, for example, Smith *et al.*, "Synthetic techniques and intermediates for polyhydroxy, dienyl lactones and mimics thereof," U.S. Patent Nos. 5,789,605 and 6,031,133; and Smith *et al.*, "Synthetic techniques

and intermediates for polyhydroxy, dienyl lactone derivatives," U.S. Patent Nos. 6,096,904 and 6,242,616 (each of which is incorporated herein by reference). The reported syntheses are long, complex, and generally not amenable for making commercial quantities of these compounds. Because there are many other polyketides of interest that are hampered by inadequate supply, and because epothilone and discodermolide could be more readily developed for therapeutic and other uses if a more adequate supply were available, a need exists for novel approaches for obtaining these important compounds.

SUMMARY

The present invention provides methods for making modular polyketide synthases and genes that encode them for the production of polyketides of defined structure. Such polyketides are useful as intermediates in the chemical synthesis of more complex polyketides, or they may be useful in their own right. The inherent stereochemical specificities of biological processes result in highly efficient production of optically-active intermediates for use in the chemical synthesis of complex polyketides. Because intermediates with complex stereochemical centers are more readily synthesized in optically-pure form using these biological strategies, polyketides may be chemically synthesized more simply and economically by these methods.

In one aspect of the invention, a method for designing a gene for making a particular polyketide compound is provided. The method comprises:

defining the compound as a sequence of two-carbon units;

comparing the two-carbon unit sequence of the compound with a database of naturally occurring PKS structures wherein each database PKS structure is also described as a sequence of two-carbon units;

for each two-carbon unit of the compound, searching the database for a matching two-carbon unit;

for each two-carbon unit of the compound for which a match was found in the database, associating a PKS gene fragment corresponding to the matched database two-carbon unit; and,

designing a new gene capable of producing said compound wherein the gene includes the PKS gene fragments associated with the matched database two-carbon units.

In a second aspect of the invention, genes encoding novel polyketide synthases (PKSs) which catalyze the formation of desired polyketides are provided. These genes comprise a collection of fragments of natural PKS genes, each fragment encoding at least a module of a PKS, or the ketosynthase, acyltransferase, and acyl-carrier protein domains of a module, capable of catalyzing the formation of a designated 2-carbon unit in the desired polyketide. Said gene fragments may be genetically engineered so as to alter the domain content of the resulting PKS module, so as to provide the desired polyketide. In preferred embodiments, the PKS gene fragments are associated with a coding sequence for a terminal thioesterase domain, and are placed in expression vectors.

In another aspect of the invention, the genes encoding novel PKSs are introduced into host cells which support the production of the desired polyketides during fermentation. In preferred embodiments, the host cells either do not naturally produce polyketides or have had their native PKS genes deleted. In particularly preferred embodiments, the host cells are *Streptomyces coelicolor, Streptomyces lividans, Streptomyces fradiae, Saccharopolyspora erythraea, Escherichia coli, Myxococcus xanthus,* or *Saccharomyces cerevesiae.*

In another aspect of the invention, the novel polyketides produced from the above host cells are provided.

In another aspect, the present invention provides a method for making a first compound useful in synthesizing a second compound, wherein said second compound contains four or more chiral centers, and said first compound contains two or more chiral centers, said method comprising expressing in a recombinant host cell a recombinant, non-naturally occurring polyketide synthase that produces said first compound. In preferred embodiments, the first compound contains at least 3 chiral centers, and the second compound contains at least 5 chiral centers. In a particularly preferred embodiment, the second compound contains at least 10 chiral centers. In a preferred embodiment, said first and second compounds are polyketides. The recombinant, non-naturally occurring PKS can be either a portion of a naturally occurring PKS gene or can be composed of portions of two or more naturally occurring PKS genes. The portions of the two PKS genes can each

comprise two or more extender modules. In a preferred embodiment, the second compound is a naturally occurring polyketide, and the non-naturally occurring recombinant PKS is derived from one or more PKS that does or do not produce the second compound.

In another aspect of the invention, a combination of biological and chemical methods for the synthesis of epothilone and epothilone analogs is provided. Intermediate compounds and methods for making the same are provided that are used as starting materials in the chemical synthesis of epothilones.

In another aspect of the invention, a combination of biological and chemical methods for the synthesis of discodermolide and discodermolide analogs is provided. Intermediate compounds and methods for making the same are provided that are used as starting materials for use in the chemical synthesis of discodermolide.

Definitions

Listed below are definitions of various terms used to describe this invention. These definitions apply to the terms as they are used throughout this specification, unless otherwise stated in specific instances, either individually or as part of a larger group.

The term "polyketide" refers to a compound that can be derived by the decarboxylative condensation of a succession of malonyl thioester extender units onto a starting acyl thioester. The malonyl thioesters may be optionally substituted, for example, methylmalonyl, ethylmalonyl, methoxymalonyl, hydroxymalonyl, and the like. Examples of starting acyl thioesters includes but is not limited to alkanoates such as acetyl, propionyl, butyryl, isobutyryl, *sec*-valeryl, and the like; cycloalkanoates such as cyclohexanoyl; alkenoates such as acryloyl and crotonoyl; cycloalkenoates, such as cyclohexenoyl; and aryl, such as benzoyl, thiazolyl, and the like. After condensation, the extender units may be further modified by redox chemistry, methylation, and other transformations. Polyketides may be either of natural origin and produced by naturally-occurring polyketide synthases, may be the products of genetically-engineered polyketide synthases either *in vivo* or *in vitro*, or may be produced by chemical synthesis. When produced by chemical synthesis, methods other than the decarboxylative condensation of a succession of malonyl thioester extender units onto a starting acyl thioester may be employed for production of polyketides.

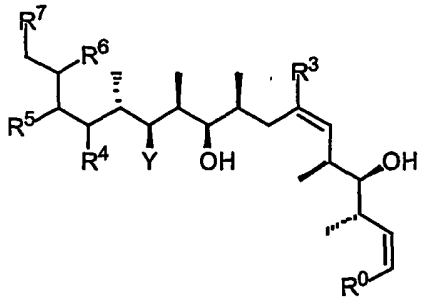
The term "polyketide synthase" ("PKS") refers to an enzyme catalyzing the biosynthesis of a polyketide. The term "modular PKS" refers to a class of PKS wherein each step in the biosynthesis of a complex polyketide is catalyzed by a separate domain of the enzyme, and said domains are arranged in a predictable order along the polypeptide chain. Examples of naturally-occurring polyketide synthases include but are not limited to those involved in the biosynthesis of erythromycin (ery), megalomicin (meg), pikromycin (pik), narbomycin (nar), oleandomycin (ole), lankamycin (lkm), FK506 (506), FK520 (asc), rapamycin (rap), epothilone (epo), tylosin (tyl), spiramycin (spm), rosamicin (rsm), geldanamycin (gdm), pimaricin (pim), FR008 (fr8), candicidin (can), avermectin (avr), tartralone (tar), borophycin (bor), aplasmomycin (apl), boromycin (brm), discodermolide (dsc), and the like.

The term "recombinant" refers to genes, proteins, or organisms which have been genetically engineered. An example of a recombinant gene is a DNA sequence which has been cloned from its original source and optionally modified so as to alter the coding sequence. An example of a recombinant protein is a protein which is expressed from a recombinant gene. An example of a recombinant organism is an organism which contains recombinant genes.

The term "module" refers to a contiguous segment of a PKS polypeptide containing the domains necessary for the addition and processing of a single extender unit onto the polyketide. A PKS module contains a core of three domains, including a ketosynthase, an acyltransferase, and an acyl-carrier protein domain. A module may also contain further domains involved in processing the added extender unit. A listing of the most common module types and their polyketide structural outcomes is given in Figure 8.

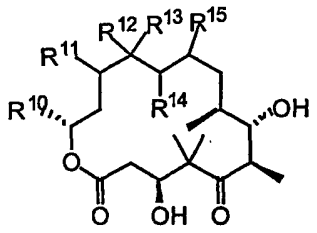
The term "domain" refers to a portion of a PKS catalyzing a single step in the biosynthesis of a polyketide. Examples of domains include but are not limited to ketosynthases (KS), acyltransferases (AT), acyl-carrier protein (ACP), ketoreductase (KR), dehydratase (DH), enoylreductase (ER), C-methyltransferase (MT), O-methyltransferase (OMT), and thioesterase (TE). The common order of domains within a module is KS – AT – [modification domains] - ACP. Modification domains occur either singly, for example as a KR or a MT, in pairs, as in DH-KR, or in triplets as in DH-ER-KR.

As used herein, the terms "discodermolides," "discodermolide compounds," and "discodermolide analogs" refer to compounds of the formula:



wherein R^0, R^1, R^2, R^3, R^4, R^5, R^6, R^7, X, and Y are described herein, and includes analogs derived therefrom that possess microtubule-stabilizing activity in one of the assays described by Bollag et al., *Cancer Research 55*: 2325-2333 (1995) (incorporated herein by reference) or in a comparable assay.

As used herein, the terms "epothilones," "epothilone compounds," and "epothilone analogs" refer to compounds of the formula:



wherein

R^{10} is alkenyl or aryl, optionally substituted with one or more groups as defined below;

R^{11} is H;

R^{12} is H;

or R^{11} and R^{12} taken together form a bond;

or R^{11} and R^{12} taken together form –O-;

R^{13} is H, alkyl, hydroxyalkyl, or fluoroalkyl;

R^{14} is H;

R^{15} is H;

or R^{14} and R^{15} taken together form a bond;

or R^{15} and R^{15} taken together form –O-;

and includes analogs derived therefrom that possess microtubule-stabilizing activity in one of the assays described by Bollag et al., *Cancer Research 55*: 2325-2333 (1995) (incorporated herein by reference) or in a comparable assay.

In preferred embodiments, R^{10} is taken from the set consisting of 1-(2-methylthiazol-4yl)-propen-2-yl, 1-(2-hydroxymethylthiazol-4yl)-propen-2-yl, 1-(2-fluoromethylthiazol-4yl)-propen-2-yl, 1-(2-aminomethylthiazol-4yl)-propen-2-yl, 6-quinolyl, and 2-methylbenzothiazol-5-yl; R^{11} and R^{12} taken together form a bond; R^{13} is methyl, hydroxymethyl, dioxolan-2-ylmethyl, and fluoromethyl; R^{14} is H; R^{15} is H; or R^{14} and R^{15} taken together form a bond.

The term "alkyl" refers to straight, branched, or cyclic hydrocarbons, optionally substituted as defined below. Examples of alkyl groups include but are not limited to methyl, ethyl, propyl, isopropyl, isobutyl, cyclopropyl, cyclobutyl, cyclopenty, cyclohexyl, and the like, including substituted forms thereof.

The term "alkenyl" refers to an straight, branched, or cyclic hydrocarbon group containing at least one carbon-carbon double bond, optionally substituted as defined below. Examples of alkenyl groups include but are not limited to vinyl, allyl, cyclohexenyl, and the like, including substituted forms thereof.

The term "alkynyl" refers to an straight, branched, or cyclic hydrocarbon group containing at least one carbon-carbon triple bond, optionally substituted as defined below. Examples of alkynyl groups include but are not limited to ethynyl, propargyl, and the like, including substituted forms thereof.

The term "aryl" refers to an aromatic moiety including heteroaryls having one or more heteroatoms such as N, O, and S, optionally substituted as defined below. Examples of aryl groups include but are not limited to phenyl, pyridyl, pyrimidinyl, pyrrolyl, pyrrazolyl, triazolyl, tetrazolyl, furyl, isoxazolyl, oxazolyl, imidazolyl, thiazolyl, thienyl, indolyl, indazolyl, quinolyl, isoquinolyl, quinoxalyl, phthaloyl, phthalimidoyl, benzimidazolyl, benothiazolyl, benzofuryl, and the like, including substituted forms thereof.

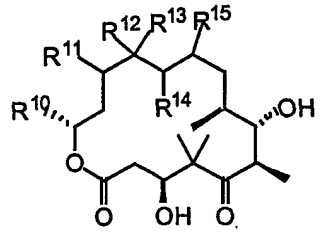
The "alkyl," alkenyl," "aryl," and other moieties may optionally be substituted with one or more substituents. Illustrative examples of substituents include but are not limited to alkyl, alkenyl, alkynyl, aryl, halogen (F, Cl, Br, I); trifluoromethyl; trifluoromethoxy; hydroxy; alkoxy; cycloalkoxy; hetoercyclooxy; oxo; alkanoyl (-C(=O)-alkyl); aryloxy; alkanoyloxy; amino; alkylamino; arylamino; aralkylamino; cycloalkylamino; heterocycloamino; disubstituted amines in which the two amino substituents are selected from alkyl, aryl, or aralkyl; alkanoylamine; aroylamino; aralkanoylamino; substituted alkanoylamino; substituted arylamino; substituted aralkanoylamino; thiol; alkylthio; arylthio; aralkylthio; cycloalkylthio; heterocyclothio; alkylthiono; arylthiono; aralkylthiono; alkylsulfonyl; arylsulfonyl; aralkylsulfonyl; sulfonamido (e.g., SO_2NH_2); substituted sulfonamido; nitro; cyano; carboxy; carbamyl (e.g., $CONH_2$); substituted carbamyl (e.g., -C(=O)NRR' where R and R' are each independently hydrogen, alkyl, aryl, aralkyl and the like); alkoxycarbonyl, aryl, substituted aryl, guanidino, and heterocyclo such as indoyl, imidazoly, furyl, thienyl, thiazolyl, pyrrolidyl, pyridyl, pyrimidyl and the like. Where applicable, the substituent may be further substituted such as with halogen, alkyl, alkoxy, aryl, or aralkyl and the like. Particularly preferred examples of substituted alkyls include fluoromethyl and fluoroethyl. Particularly preferred examples of substituted aryls include 2-methyl-4thiazolyl, 2-(hydroxymethyl)-4-thiazolyl, 2-(fluoromethyl)-4-thiazolyl, and 2-(aminomethyl)-4-thiazolyl.

The term "hydroxy protecting group" refers to groups known in the art for such purpose. Commonly used hydroxy protecting groups are disclosed, for example, in T. H. Greene and P.G. M. Wuts, Protective Groups in Organic Synthesis, 2nd edition, John Wiley & Sons, New York (1991), which is incorporated herein by reference. Illustrative hydroxyl protecting groups include but not limited to tetrahydropyranyl (THP); benzyl; 4-methoxybenzyl (PMB); methylthiomethyl; ethythiomethyl; pivaloyl; phenylsulfonyl; triphenylmethyl; trisubstituted silyl such as trimethyl silyl (TMS), triethylsilyl (TES), tributylsilyl, tri-isoprylsilyl (TIPS), t-butyldimethylsilyl (TBS), tri-t-butylsilyl, methyldiphenylsilyl, ethyldiphenylsily, t-butyldiphenylsilyl and the like; acyl and aroyl such as acetyl (Ac), pivaloylbenzoyl (Piv), 4-methoxybenzoyl, 4-nitrobenzoyl and aliphatic acylaryl and the like. All hydroxyl groups of compounds described herein may optionally be protected with a hydroxy protecting group.

In addition to the explicit substitutions at the above-described groups, the inventive compounds may include other substitutions where applicable. For example, the discodermolide backbone (e.g., C-1 through C-24) or backbone substituents may be additionally substituted (*e.g.*, by replacing one of the hydrogens or by derivatizing a non-hydrogen group) with one or more substituents such as C_1-C_5 alkyl, C_1-C_5 alkoxy, phenyl, or a functional group. Illustrative examples of suitable functional groups include but are not limited to alcohol, sulfonic acid, phosphine, phosphonate, phosphonic acid, thiol, ketone, aldehyde, ester, ether, amine, quanternary ammonium, imine, amide, imide, imido, nitro, carboxylic acid, disulfide, carbonate, isocyanate, carbodiimide, carboalkoxy, carbamate, acetal, ketal, boronate, cyanohydrin, hydrozone, oxime, hydrazide, enamine, sulfone, sulfide, sulfenyl, and halogen.

Epothilone Compounds of the Invention

In one aspect of the invention, epothilone analogs of the following formula are provided:



wherein

R^{10} is alkenyl or aryl;

R^{11} is H;

R^{12} is H;

or R^{11} and R^{12} taken together form a bond;

or R^{11} and R^{12} taken together form –O-;

R^{13} is H, alkyl, hydroxyalkyl, or fluoroalkyl;

R^{14} is H;

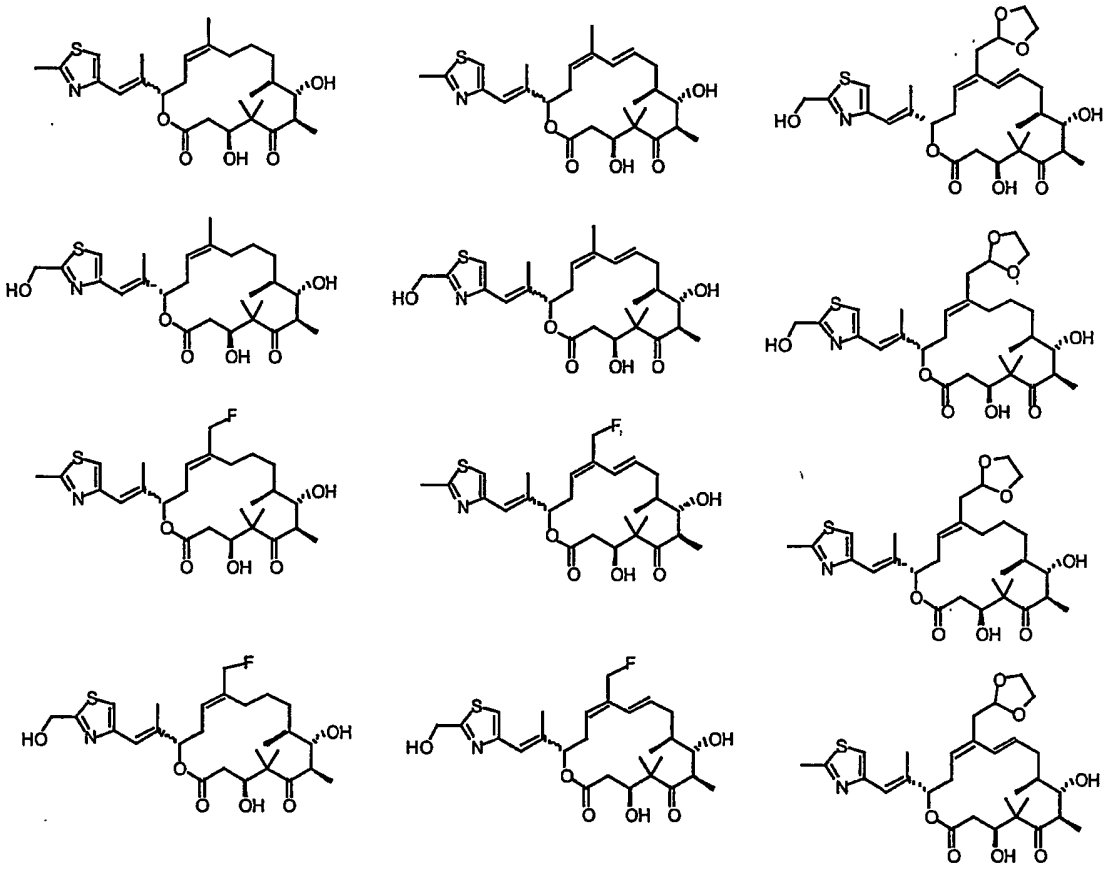
R^{15} is H;

or R^{14} and R^{15} taken together form a bond;

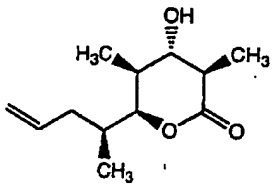
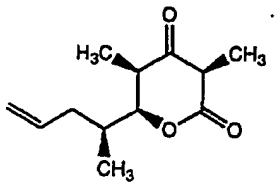
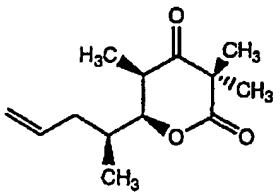
or R^{14} and R^{15} taken together form –O-.

In preferred embodiments, R^{10} is taken from the group consisting of 1-(2-methylthiazol-4yl)-propen-2-yl, 1-(2-hydroxymethylthiazol-4yl)-propen-2-yl, 1-(2-fluoromethylthiazol-4yl)-propen-2-yl, 1-(2-aminomethylthiazol-4yl)-propen-2-yl, 6-quinolyl, and 2-methylbenzothiazol-5-yl; R^{11} and R^{12} taken together form a bond; R^{13} is methyl, hydroxymethyl, dioxolan-2-ylmethyl, and fluoromethyl; R^{14} is H; R^{15} is H; or R^{14} and R^{15} taken together form a bond.

In a particularly preferred embodiment, the epothilone analog is selected from the group consisting of:



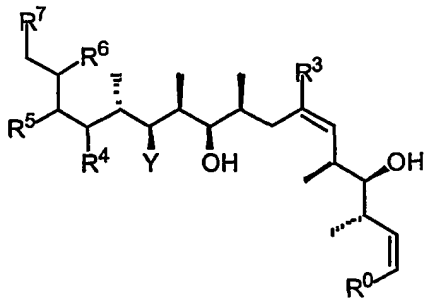
In another aspect of the invention, intermediates leading to the synthesis of the above epothilone analogs are provided. In preferred embodiments, these intermediates are taken from the group consisting of:

, , and .

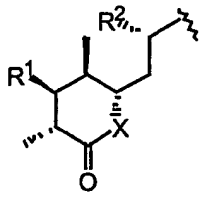
Discodermolide Compounds of the Invention

In one aspect of the present invention, novel discodermolide compounds are provided of the formula:



where

R^0 is C1-C8 alkyl, C1-C8 alkenyl, C1-C8 alkynyl, aryl, 2-phenylethyl, 2-(3-hydroxyphenyl)ethyl, or a group of the formula



wherein R^1 and R^2 are each independently hydrogen, hydroxyl, or a hydroxyl protecting group; and X is O, NH, or N-alkyl;

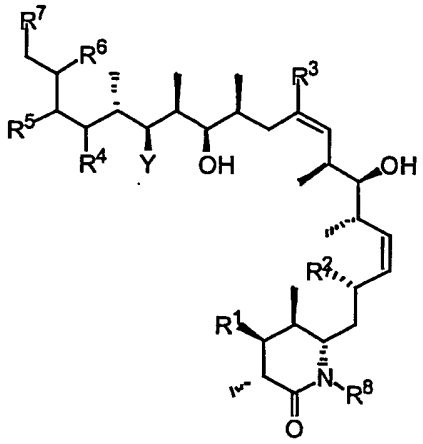
R^3 is hydrogen, C_1-C_{10} alkyl or aryl;

R^4, R^5, R^6, and R^7 are each hydrogen, or R^4 and R^5 together form a double bond and R^6 and R^7 together form a double bond; and

Y is hydroxyl, amino, -OC(=O)NH_2 or -NHC(=O)NH_2,

with the proviso that when R^3 is hydrogen or C_1-C_6 alkyl that: (i) at least one of R^1 and R^2 is not hydroxyl, or (ii) R^4, R^5, R^6, and R^7 are each hydrogen, or (iii) X is nitrogen, or (iv) Y is hydroxyl, amino, or -NHC(=O)NH_2, or (v) any combination of (i) through (iv).

In another embodiment of the present invention, compounds are provided of the formula



wherein

R^1 and R^2 are each independently hydrogen, hydroxyl, or a hydroxyl protecting group;

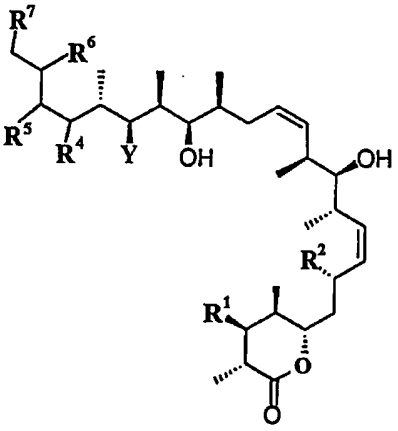
R^3 is hydrogen, C_1-C_{10} alkyl or aryl;

R^4, R^5, R^6, and R^7 are each hydrogen, or R^4 and R^5 together form a double bond and R^6 and R^7 together form a double bond;

R^8 is H or C_1-C_8 alkyl; and,

Y is hydroxyl, amino, -OC(=O)NH_2 or -NHC(=O)NH_2.

In another embodiment of the present invention, compounds are provided of the formula



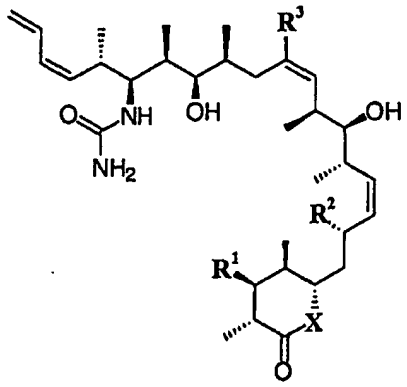
wherein

R^1 and R^2 are each independently hydrogen, hydroxyl, or a hydroxyl protecting group;

R^4, R^5, R^6, and R^7 are each hydrogen, or R^4 and R^5 together form a double bond and R^6 and R^7 together form a double bond; and,

Y is hydroxyl, amino, -OC(=O)NH_2 or -NHC(=O)NH_2, provided that at least one of R^1 and R^2 is not hydroxyl.

In yet another embodiment of the present invention, compounds are provided of the formula



wherein

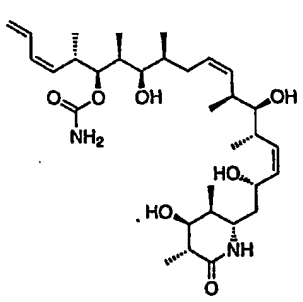
R^1 and R^2 are each independently hydrogen, hydroxyl, or a hydroxyl protecting group;

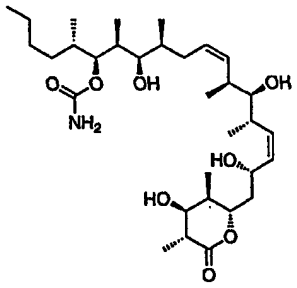
R^3 is hydrogen, C_1-C_{10} alkyl or aryl;

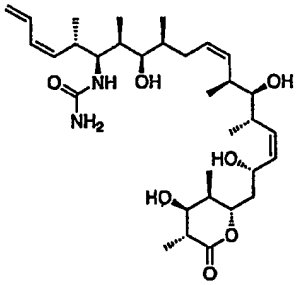
X is O, NH, or N-alkyl; and,

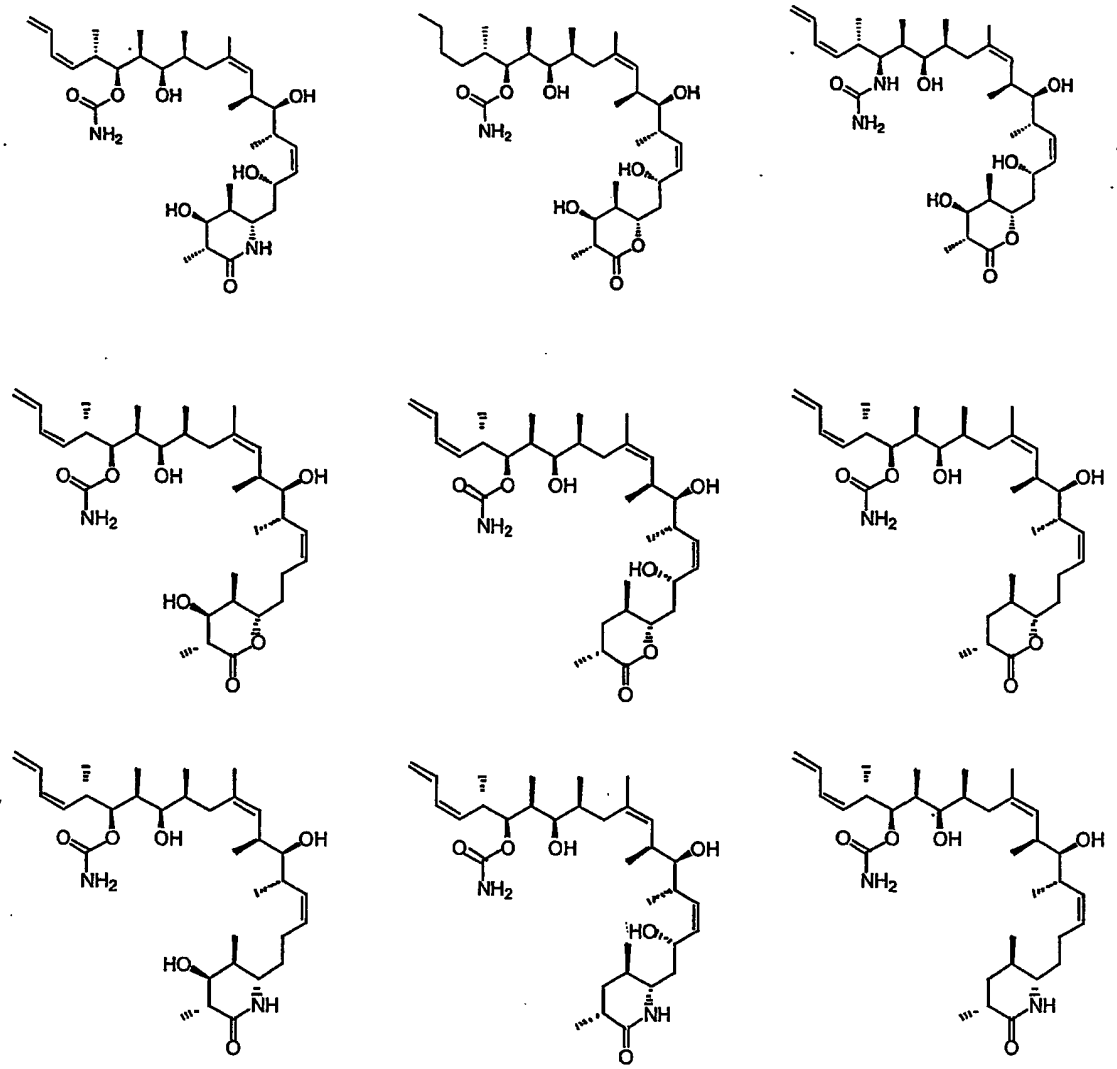
Y is hydroxyl, amino, -OC(=O)NH_2 or -NHC(=O)NH_2,

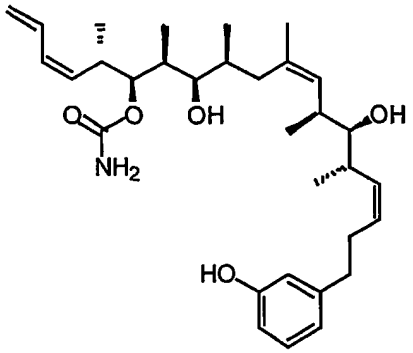
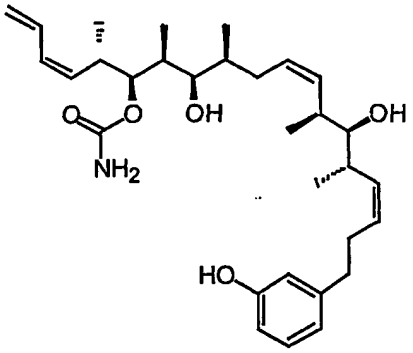
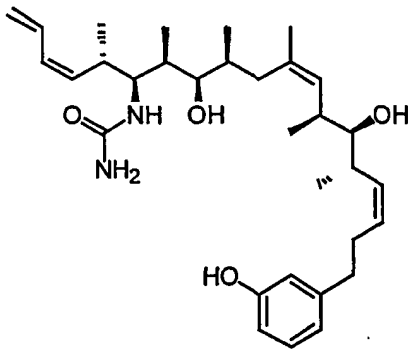
Particularly preferred embodiments of the present invention include but are not limited to:







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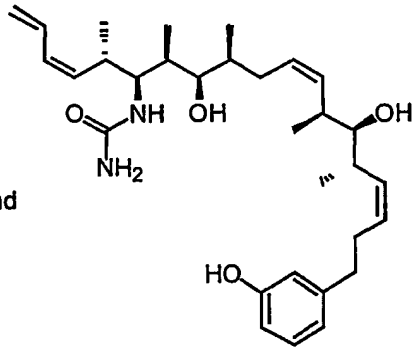
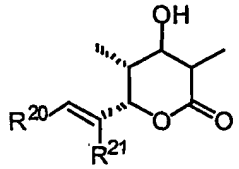
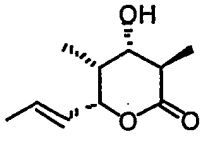
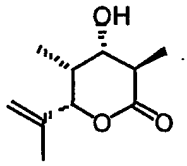
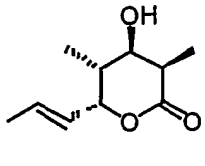
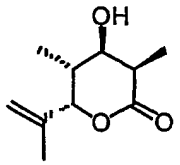


In another aspect of the invention, intermediates leading to the synthesis of the above discodermolide analogs are provided.

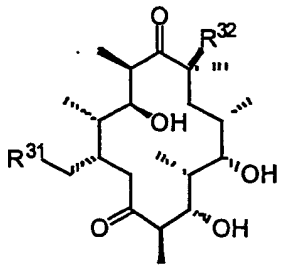
In one embodiment, intermediates of the formula



are provided, wherein R^{20} is hydrogen, alkyl, or aryl; and R^{21} is hydrogen or alkyl. Preferred embodiments include but are not limited to:




and


In another embodiment, compounds of the formula



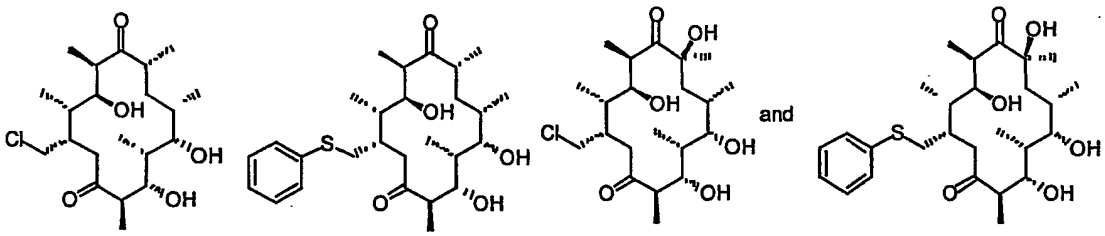
are provided, wherein

R^{31} is hydrogen, alkyl, alkenyl, halogen or phenylthio; and

R^{32} is hydrogen or hydroxy;

with the proviso that when R^{31} is hydrogen or alkyl, that R^{32} can not be hydrogen.

Preferred embodiments include but are not limited to:



Genes and Enzymes of the Invention

In one aspect of the invention, novel polyketide synthase genes are constructed by combining fragments of naturally occurring PKS genes, along with a DNA sequence encoding a terminal thioesterase domain located at the end of the sequence encoding the last extender module, and cloning them into suitable expression vectors behind functional promoters. Examples of suitable expression vectors for actinomycete host cells, such as *Streptomyces*, include both autonomously replicating vectors and integrating vectors which insert into the host chromosome. Preferred examples of replicating vectors for *Streptomyces* include those based on the SCP2* replicon, such as pRM1 and pRM5. Preferred examples of integrating vectors include but are not limited to vectors containing sequences allowing for integration at phage attachment sites. Particularly preferred examples of integrating vectors for *Streptomyces* are those using the ϕC31 phage sequences, including but not limited to pSET and pSAM. A listing of suitable actinomycete vectors is found in Kieser *et al*, "Practical Streptomyces Genetics," (John Innes, Norwich, 2000), which is incorporated herein by reference.

Expression of the constructed PKS genes in a suitable host results in production of a functional PKS. Suitable actinomycete host cells include but are not limited to members of the genera *Streptomyces, Saccharopolyspora,* and *Micromonospora.* Preferred examples of actinomycete host cells are members of the genera *Streptomyces* and *Saccharopolyspora.* Particularly preferred actinomycete host cells are *Streptomyces coelicolor, Streptomyces lividans, Streptomyces fradiae,* and *Saccharopolyspora erythraea.* Suitable host cells typically have had their native PKS genes deleted or otherwise rendered non-functional, for example through mutagenesis, according to the methods described in Khosla *et al.*, "Recombinant production of novel polyketides" U.S. Patent 5,830,750 (incorporated herein by reference). Particularly preferred examples of non-actinomycete host cells include suitably prepared *Escherichia coli, Saccharomyces cerevesiae,* and *Myxococcus xanthus.* The preparation and use of *Escherichia coli* and *Saccharomyces cerevesiae* host cells is described in Santi *et al.*, "Heterologous production of polyketides," PCT publication no. WO01/31035 and in Pfeifer & Khosla, "Biosynthesis of polyketide substrates," PCT publication no. WO01/27306 (both of which are incorporated herein by reference). The use of *Myxococcus xanthus* as a host cell is described in Julien *et al.*, "Producing epothilone and epothilone derivatives," PCT publication no. WO00/31247 (incorporated herein by reference).

In one embodiment, n-module PKS genes are constructed by fusing contiguous coding sequences for modules from a natural PKS to a coding sequence for terminal TE domain to produce a new PKS gene. Such novel PKS enzymes and the genes that encode them are herein designated:

(source)[module(1)]-[module(2)]-...- [module(n-1)]-[module(n)]-TE.

As an example, a two-module PKS comprising modules 5 and 6 of the erythromycin PKS genes along with the TE domain is herein designated:

(ery)[module(5)]-[module(6)]-TE.

In another embodiment, PKSs comprising modules are construced by fusing modules or contiguous sets of modules obtained from different natural PKS genes. As an example, a three-module PKS comprising module 1 of the erythromycin PKS fused with modules 5 and 6 of the narbomycin PKS and a TE is herein designated:

(ery)[module(1)]-(nar)[module(5)]-[module(6)]-TE.

In another embodiment of the invention, PKSs comprising modules which have been mutated so as to alter the complement of domains contained within the modules are constructed. Such PKSs are constructed either using modules from the same or from different natural PKS genes. Domains which have been inactivated through mutagenesis, but not deleted, are indicated by the symbol "^{0}."As an example, a three-module PKS comprising module 1 of the erythromycin PKS, wherein the KS domain has been inactivated through mutagenesis, fused with modules 5 and 6 of the narbomycin PKS and a TE is herein designated:

(ery)[module(1)-KS^{0}]-(nar)[module(5)]-[module(6)]-TE.

Domain deletions are indicated by "Δ," such that a two-module PKS comprising modules 1 and 2 of the erythromycin PKS, in which the KR domain of module 2 has been deleted, is herein designated:

(ery)[module(1)]-[module(2)-ΔKR]-TE.

Domain substitutions are indicated by a "/." Thus, a two-module PKS comprising modules 1 and 2 of the erythromycin PKS, in which the KR domain of module 2 has been replaced with the KR domain taken from module 4 of the rapamycin PKS, is herein designated:

(ery)[module(1)]-[module(2)KR/rapKR4]-TE.

Domain additions are indicated by "+," such that a two-module PKS comprising modules 1 and 2 of the erythromycin PKS, in which the MT domain from module 8 of the epothilone

PKS gene has been added without other alteration of the module domains, is herein designated:

(ery)[module(1)]-[module(2)+epoMT8]-TE.

Examination of polyketide structures reveals multiple occurrences of modules having identical functions in various PKSs. Because of this, several PKS genes may provide modules of equivalent function, and may be used interchangeably according to the present invention. Thus, while specific sources for modules are used in the description of the invention for the purposes of illustration, it is intended that modules of equivalent function from different sources may be freely interchanged in accord with the methods of the invention.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 depicts the organization of the eryAI, eryAII, and eryAIII genes that encode the PKS enzyme deoxyerythronolide B synthase ("DEBS") (which is composed of DEBS1, DEBS2, and DEBS3 protein subunits) that makes 6-deoxyerythronolide B ("6-dEB").

Figure 2 is the macrolactonization synthetic strategy developed by Danishefsky for the *de novo* synthesis of epothilone D starting from two key intermediates, a thiazole fragment and Compound A.

Figure 3 depicts the organization of a two-module PKS capable of converting Compound (2) into Compound (1).

Figure 4 is a graphical representation of the epothilone PKS.

Figure 5 illustrates the relationships between some key compounds of the invention. Single arrows indicate biological or biochemical transformations, while double arrows indicate chemical transformations. Arrows may represent multiple steps.

Figure 6 illustrates a novel protocol for the synthesis of epothilone D using Compound (10) in place of Compound A.

Figure 7 illustrates another novel protocol for the synthesis of epothilone D using Compound (1) in place of Compound A.

Figure 8 shows the polyketide structures produced by 14 common PKS modules, along with reported cases of modules with MT domains.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

Polyketides are naturally occurring compounds that are made by many organisms including fungi and mycelia bacteria. A diverse class of natural products, polyketides are classified as such because they are synthesized, at least in part, from two carbon unit building blocks through a series of Claisen type condensations by polyketide synthase ("PKS") enzymes. Two major types of PKS enzymes are modular PKS and iterative (or "aromatic") PKS. Modular PKS enzymes are typically multi-protein complexes in which each protein contains multiple active sites, each of which is used only once during carbon chain assembly and modification. Iterative PKS enzymes are typically multi-protein complexes in which each protein contains only one or at most two active sites, each of which is used multiple times during carbon chain assembly and modification.

Polyketides made by modular PKS enzymes have a variety of biological activities and include important drugs such as erythromycin and tacrolimus (also known as FK-506). A prototypical example of modular PKS enzymes is deoxyerythronolide B synthase ("DEBS") that synthesizes 6-deoxyerythronolide B ("6-dEB"), an erythromycin precursor. The organization of these eryA genes which encode DEBS and/or methods for their manipulation are described in U.S. Patent Nos. 5,712,146 and 5,824,513, 6,004,787, 6,060,234, and 6,063,561 each of which is incorporated herein by reference.

Modular PKS enzymes are so termed because they are organized into distinct units (or modules) that ultimately control the structure of a discrete two-carbon portion of the polyketide the structure. PKS enzymes generally contain (i) a loading domain, (ii) a number of extender modules, (iii) and a releasing domain (which is also called a thioesterase domain). The two-carbon units are of the general formula (R-C(=O)) from which polyketides are synthesized and are generally referred to as starter units or extender

units depending on whether the two carbon unit initiates the synthesis of the polyketide or extends (adds to) the growing polyketide chain during synthesis. Starter units bind to the loading domain and initiate the polyketide synthesis and (ii) extenders bind to the extender modules and extend the polyketide chain. Starter units and extender units are typically acylthioesters, most commonly acetyl-CoA, propionyl-CoA, and the like for starter units and malonyl-CoA, methylmalonyl-CoA, methoxymalonyl-CoA, hydroxymalonyl-CoA, ethylmalonyl-CoA, and the like for extender units.

Each module of a modular PKS contains three core domains needed for polyketide synthesis, an acyltransferase (AT) responsible for selecting and binding the appropriate extender unit, an acyl-carrier protein (ACP) responsible for carrying the growing polyketide chain, and a β-ketoacylsynthase (KS) responsible for condensing the extender unit onto the growing polyketide chain. Together, these core domains add a 2-carbon β-ketothioester onto the growing end of the polyketide chain.

In addition, a module may contain a set of reductive cycle domains responsible for modifying the β-ketone produced by the core domains. If present, a ketoreductase (KR) domain reduces the β-ketone to an alcohol of defined stereochemistry. If present with a KR, a dehydratase (DH) domain eliminates the alcohol produced by the KR to form an alkene. If present with a DH and a KR, an enoylreductase (ER) domain reduces the alkene produced by the DH to form a saturated alkane. Other types of modification domains, such as methyltransferase (MT) domains, can also be present in a module. MT domains add a methyl group, typically from S-adenosylmethionine (SAM), to the α-carbon of the newly-added 2-carbon unit, and O-methyltransferase domains (OMT) add the methyl group instead to the oxygen atom of the enol form of the β-ketothioester to form a methyl vinyl ether or to the alcohol resulting from the action of a KR domain so as to form a methyl ether. Examples of known modules with MT or OMT domains are shown in Figure 8.

A listing of the 14 functional modules possible using only malonyl- and methylmalonyl-specific AT domains and only KR, DH, and ER modification domains is given in Figure 8. Other modules are known, as AT specificities for ethylmalonyl- and methoxymalonyl-extender units are also known.

The order of modules as they are encoded within the gene appears to follow the order in which they function in the biosynthesis. The order of domains within a module, while conserved between PKSs, does not appear to follow the order in which they function in the biosynthesis. The boundaries of domains are readily recognized due to the high homology that exists between the many known examples of PKS genes.

Figure 1 is a schematic representation of the DEBS enzyme, which is responsible for the biosynthesis of the polyketide core of erythromycin. DEBS is the prototypical modular PKS, and is a dimer of three proteins, DEBS1, DEBS2, and DEBS3. The organization of the genes that encode DEBS and methods for their manipulation are described in U.S. Patents 5,712,146, 5,824,513, 6,004,787, 6,060,234, and 6,063,561, each of which is incorporated herein by reference.

As shown by Figure 1, DEBS1 comprises the loading domain and the first and second extender modules. DEBS2 comprises the third and fourth extender modules. DEBS3 comprises the fifth and sixth extender modules and the releasing domain. Modular PKSs are commonly dimers of multiple polypeptides, although the division of number of modules between the polypeptides is highly variable.

The DEBS loading domain consists of a special AT domain that binds the starter unit and an acyl carrier protein ("ACP"). Synthesis of 6-dEB progresses as follows. The loading domain AT recognizes propionyl CoA and binds the acyl group (the propionyl group) though a serine residue (Ser-O-C(=O)-CH_2CH_3). The loading domain AT transfers the acyl group to the loading domain ACP which binds the acyl group through a cysteine residue (Cys-S-C(=O)-CH_2CH_3). Concurrently, each of the six extender modules recognizes methylmalonyl CoA and transfers the corresponding acyl group to the ACP of that module (Cys-S-C(=O)-$CH(CH_3)$C(=O)OH). Synthesis commences when the loading domain ACP transfers the propionyl group to the KS of the first extender module which positions the group for the condensation reaction with the methylmalonyl thioester of the first extender module ACP (with a concomitant elimination of CO_2). After the condensation reaction, the first extender ACP now has bound a β-ketothioester (-Cys-S-*C(=O)-CH(CH_3)*C(=O)CH_2CH_3 where the italicized portion represents the two carbon unit

from the methyl malonyl CoA and the bold portion represents the two carbon unit from the propionyl CoA).

Because of the presence of the KR domain in module 1, the keto group of the β-ketothioester is modified into an alcohol. This modified precursor is depicted bound to the first extender module ACP in Figure 1. As the synthesis progress, this precursor is transferred to second extender module KS where the recently-extended acyl group is positioned for the condensation reaction with the methylmalonyl thioester bound to the second extender module ACP. The polyketide chain attached to the second extender module ACP also shows the previous keto group reduced to an alcohol. Because the third extender module KR domain is inactive, the polyketide chain attached to the third extender module ACP shows an unmodified keto group. The polyketide chain attached to the fourth extender module shows a fully saturated two-carbon unit due to the presence of a KR, DH and ER domains. As with the first and second extender module ACPs, the polyketide chains attached to the fifth and sixth extender modules depict an alcohol moiety due to the presence of the respective KR domains. Finally, the polyketide synthesis terminates when it is released from the PKS enzyme by the TE domain and forms a cyclic ester (also called a lactone or macrolactone).

Other modular PKS enzymes synthesize polyketides in a similar manner. The variations in structure of naturally occurring polyketides result from differences in the identities of the starter unit, the number of extender modules, differences in the identity of the extender unit that is recognized by each extender module, and the presence or absence of additional functionalites (e.g., KR, DH, ER, MT) in each extender module. The same type of variations can be engineered using recombinant techniques to produce derivatives of naturally occurring polyketides and entirely novel polyketides. Recombinant methods for manipulating modular PKS genes are described, for example, by U.S. Patent Nos. 5,672,491; 5,712,146; 5,830,750; and 5,843,718; and in PCT patent publication Nos. 98/49315 and 97/02358, each of which is incorporated herein by reference.

The present invention takes advantage of these techniques to provide a general process for making any polyketide that is independent of a host organism or a naturally occurring PKS gene. The method generally comprises:

Comparing the structure of a polyketide or polyketide-like compound to be made biologically with a library of PKS structures;

identifying at least one library structure having a common element with the structure of said compound;

associating a PKS gene for each identified library structure; and,

designing a new gene capable of producing said compound wherein the new gene includes at least a portion of one PKS gene corresponding to an identified library structure. A method for implementing such a method using a computer has been previously described in PCT Patent Application No. US01/17352 filed on May 29, 2001 entitled Design of Polyketide Synthase Genes by inventors Daniel V. Santi *et al.*, which is incorporated herein by reference.

In preferred embodiments, the method further involves dividing the target polyketide structure into two-carbon units and for each two-carbon unit, identifying an extender module that would provide the corresponding structure. The method comprises:

describing the target compound as sequence of two-carbon units;

comparing the two-carbon unit sequence of the target compound with a database of naturally occurring PKS structures wherein each database PKS structure is also described as a sequence of two-carbon units;

for each two-carbon unit of the target compound, searching the database for a matching two-carbon unit;

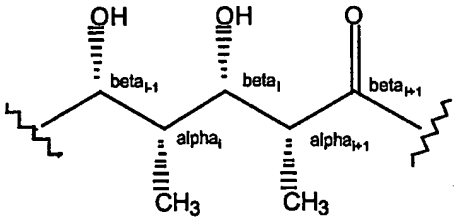
for each two-carbon unit of the target compound for which a match was found in the database, associating a PKS gene fragment corresponding to the matched database two-carbon unit; and,

designing a new gene capable of producing said compound wherein the gene includes the PKS gene fragments associated with the matched database two-carbon units.

Ideally, each two-carbon unit of the target compound will find matching counterparts in the library or database of naturally occurring polyketides. When this is the case, the identified PKS gene fragments or extender modules are then assembled together with a releasing domain into a gene which when expressed as a functional PKS system will make the desired product. The extender modules may be from a single PKS enzyme or multiple PKS enzymes although it is generally preferred to maximize the number of

consecutive modules that are taken from a single PKS enzyme. In this manner, genes are constructed that have the minimum number of non-native module boundaries to avoid undue disruption to the PKS enzyme structure.

For simplicity the general method will be illustrated for a polyketide fragment having the following structure



The fragment includes two two-carbon units, i and i+1. The i-th extender module attaches the two carbon unit whose backbone carbons are designated as alpha$_i$ and beta$_i$ and the second extender module attaches the two carbon unit whose backbone carbons are designated as alpha$_{i+1}$ and beta$_{i+1}$.

The components that are required for extender modules i and i+1 are analyzed by examining the groups off of the alpha and beta carbons. As described previously, differences in the substituents off the carbons at the alpha positions are due to the differing extender module AT specificities for acylthioesters. Two groups that are commonly found as substituents off the alpha carbon are methyl and hydrogen which are due to the extender module AT's specificity for methylmalonyl CoA and malonyl CoA respectively. Similarly, other groups include ethyl, hydroxy, and methoxy which generally are due to the extender module AT's specificity for ethylmalonyl CoA, hydroxymalonyl CoA, and methoxymalonyl CoA respectively. The differences in the functionality associated with the beta carbons are due to absence or the presence of one or more modification domains as described above.

In the above fragment, the i-th extender module requires an AT that is specific for methyl malonyl CoA (due to the methyl group off alpha$_i$) and a KR due to the presence of an alcohol moiety off the beta$_{i-1}$ carbon. Consequently, the i-th extender module would comprise KS, AT, KR, and ACP domains. The i+1 extender module also requires an AT that is specific for methyl malonyl CoA (due to the methyl group off alpha$_{i+1}$). Because of the hydroxyl group off beta$_i$, the i+1 extender module also would comprise KS, AT, KR

and ACP domains. The keto group off the $beta_{i+1}$ carbon indicates that the next extender module (or the i+2 extender module) will not require any additional functionality enzymes. In other words, the i+2 extender module would only comprise KS, AT, and ACP domains. A module consisting of KS, AT, and ACP domains is termed a minimal module.

Many different strategies can be used to make the portion of a gene that can synthesize the above fragment. In one embodiment, extender modules from known PKS genes may be used. For example, the structure of the above fragment is identical to the portion of the erythromycin that is synthesized by extender modules 5 and 6 of the erythromycin PKS gene. See e.g., Figure 1. As a result, the gene that encodes for extender modules 5 and 6 may be used without modification.

Exact matches of a particular fragment structure to consecutive modules of a single PKS are more likely where the number of two-carbon units that comprise the fragment is small. If such an exact match were not found, then a gene for making the desired fragment may be constructed in accordance with the methods of the invention from multiple PKS genes. For example, if a desired fragment needs to be constructed with six extender modules, it may be constructed by combining two extender modules from a first PKS and four modules from a second PKS. Although the genes that encode the modules are manipulated to allow the modules from the two different PKSs work together, the individual module's AT specificity or the number of functionality enzyme domains is not usually altered. Where possible, it is generally preferred to use the maximum number of consecutive modules from a single PKS gene. In other words, genes are constructed that have the minimum number of non-native module boundaries to avoid undue disruption to the resulting PKS enzyme structure as well as minimize the number of cloning manipulations that must be performed in order to assemble the genes.

Because module organization may be inferred from the final polyketide structure, practice of the present invention is amenable even in situations where the component PKS gene itself has not yet been identified. For example, if the DEBS gene were not known, the fact that the desired fragment is identical to the portion of 6-dEB that is encoded by extender modules 5 and 6 of the DEBS PKS gene may still be determined from the general organization of modular PKS genes. If the portion of the DEBS gene is determined to be

the most suitable, then a probe may be constructed from conserved regions of known PKS genes to find and sequence the DEBS gene. In general, intact PKS genes are readily retrievable because the genes coding for the core components of the PKS (loading domain, extender modules, and releasing domain) as well as the genes for the tailoring enzymes are generally contiguous. Once the desired PKS gene is obtained, its organization may be determined and the coding sequences for its modules used as described herein.

In another embodiment, the required extender modules are engineered by modifying one or more modules of a particular PKS. An illustrative example of a modification is changing the AT specificity of a module. Another modification is changing the number of functionality domains by either adding or deleting one or more functionality domains. In a variation of the latter, the function of an existing functionality domain may be inactivated. In each of these cases, the method comprises

describing the compound as sequence of two-carbon units;

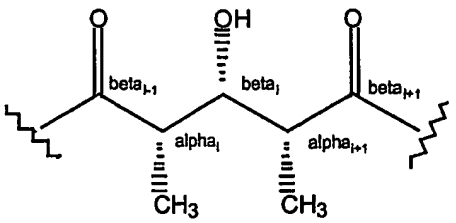
comparing the two-carbon unit sequence of the compound with a database of naturally occurring PKS structures wherein each database PKS structure is also described as a sequence of two-carbon units;

identifying a database PKS structure having the most number of matching consecutive two-carbon units and having at least one non-matching two-carbon unit;

identifying a PKS gene responsible for making the database PKS structure;

determining one or more alterations to the PKS gene in the portion responsible for making the at least one non-matching two-carbon unit to make a matching two-carbon unit.

An illustration of this embodiment also benefits from a simplified example. Using the prototypical PKS gene, extender modules 5 and 6 of the DEBS PKS are modified to correspond to the following fragment structure:



This fragment differs from the fragment used in an earlier example in that there is a keto instead of a hydroxyl group off the beta$_{i-1}$ carbon. As a result, a gene that would correspond to the fragment would include the following sequence: KS_i, AT_i, ACP_i, KS_{i+1},

AT_{i+1}, KR_{i+1} and ACP_{i+1} wherein both AT_i and AT_{i+1} possess specificities for methyl malonyl CoA. The sequence of domains for extender modules 5 and 6 of the erythromycin PKS is: KS_5, AT_5, KR_5, ACP_5, KS_6, AT_6, KR_6 and ACP_6. As a result, the sequence of domains corresponding to erythromycin PKS modules 5 and 6 needs to be modified where the function of KR_5 is inactivated. The inactivation may occur by mutating the sequence of KR_5 so that it is no longer functional or by deleting the domain all together. Although the above example is simplistic, it serves as a platform to illustrate that an existing module may be modified in a number of ways. First, its AT may be replaced with another AT having a different specificity (a malonyl CoA specific AT for a methyl malonyl CoA specific AT) or an existing AT may be mutated to possess a different specificity. Second, existing functionality domains may be inactivated as the KR_5 in the above example. Alternatively, functionality domains may be added. For example, a KR may be added to a minimal module comprising KS, AT, and ACP. Similarly, a DH or a DH and an ER may be added to a domain comprising KS, AT, KR, and ACP

Due to the simplicity of this example, the novel gene was designed using components derived from a single PKS gene. For more complicated compounds, the novel PKS gene will likely be a chimeric gene made from at least two PKS genes that each encode a naturally occurring PKS compound. Many such PKS genes are known and are suitable for this purpose, including but not limited to the PKSs involved in the biosynthesis of erythromycin, megalomicin, picromycin, narbomycin, oleandomycin, lankamycin, FK506, FK520 (ascomycin), rapamycin, epothilone, tylosin, spiramycin, rosamicin, geldanamycin, pimaricin, FR008, candicidin, avermectin, and the like. Further, methods for identification and cloning of new PKS genes are known, as for example in Santi *et al.*, "Method for Cloning PKS Genes," PCT publication WO01/53533, published July 26, 2001 (incorporated herein by reference), and can be used to obtain fragments of PKS genes to be used according to the methods of the present invention.

According to the methods of the invention, the above described methods may be reiterated any number of times to synthesize any polyketide. The polyketide may be the final compound or may be used as a starting material for further chemical modification. Although the present invention may be used to make novel polyketides, it may also be used to make known polyketides using novel PKS genes. For example, epothilone may be made

with a chimeric gene in spite of the fact that the epothilone gene is known. However, because epothilone is naturally produced in such low yields, strategies involving chimeric genes, particularly those comprising high expressing PKS genes, may also result in high expression of the chimeric epothilone producing genes.

Epothilone

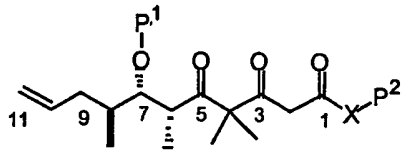
A variation of making full length polyketides is making polyketide fragments that may be used as stereochemically pure reagents in chemical syntheses. Unlike non-enzymatic reactions typical of synthetic organic chemistry, enzymatic reactions typically show essentially absolute stereoselectivity. Thus, the complex sequence of reactions performed by DEBS (ca. 27 steps) proceed with apparent absolute stereocontrol, as no stereoisomers of the product 6-deoxyerythronolide B have been identified from the enzymatic reaction.

In one embodiment of this aspect of the present invention, novel compounds and methods are used to make a previously described compound, as illustrated herein with epothilone intermediates. In other aspects of the present invention, novel compounds and methods are used to make novel intermediates for use in previously described synthetic protocols, as illustrated herein with reference to epothilones. In yet other aspects of the present invention, novel compounds and methods are used to make a compound using novel synthetic strategies as illustrated herein with reference to epothilones.. As described previously, an obstacle in the clinical evaluation of epothilones is the limited quantities that may be obtained from natural sources. Although several groups have developed *de novo* synthetic protocols for making epothilones A-D, these syntheses are complex, cumbersome, and not generally suitable for making large quantities of material. A general problem with these protocols is the difficulty in synthesizing certain precursor compounds typically due to the number and complexity of the compounds' stereocenters. In many cases, if the syntheses of these precursors were simplified, the *de novo* protocols for making polyketides such as epothilone on a commercial scale would become economically feasible.

An illustrative *de novo* synthetic protocol for making epothilone is the macrolactonization strategy outlined by Danishefsky and coworkers (Balog *et al.*, 1998, A

novel aldol condensation with 2-methyl-4-pentenal and its application to an improved total synthesis of epothilone B, *Angew. Chem. Int. Ed. Engl. 37*(19): 2675-2678, incorporated herein by reference). Figure 2 illustrates the synthetic protocol as it is applied to the synthesis of epothilone D starting from two key intermediates, the thiazole intermediate and compound A.

The formula of compound A is

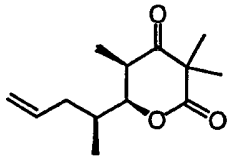


A

wherein P^1 and P^2 are H or protecting groups and X is either oxygen or sulfur. In the Danishefsky protocol, P^1 is 2,2,2-trichloroethoxycarbonyl ("Troc"), P^2 is *tert*-butyl, and X is oxygen. The C10-C11 double bond is coupled to the thiazole intermediate via Suzuki coupling. The carboxylate group at C1 is subsequently used to form the epothilone macrolactone via an intramolecular esterification. As shown by Figure 2, prior to the macrolactonization reaction, a Noyori reduction is performed to reduce the C-3 keto group derived from Compound A into an alcohol to provide the appropriate epothilone functionalities off carbons 1 through 11.

If Compound A, or an advanced precursor of Compound A comprising the stereogenic centers in Compound A, could be made biologically, many of the stereoselectivity problems associated with making epothilone would be eliminated, allowing the cost-effective synthesis of the epothilones.

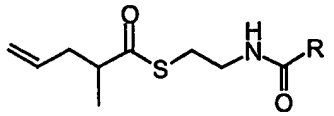
In one aspect of the invention, a compound (1) of the following formula



(1)

comprising C3-C11 of Compound A is made using an engineered polyketide synthase. In one embodiment, (1) is converted through a short series of chemical transformations into Compound A. In a further embodiment, (1) is converted more directly into epothilone.

In one embodiment of the invention, (1) is prepared by providing racemic 2-methyl-4-pentenoate N-acetylcysteamine thioester (2), a compound of the following formula



(2)

to an engineering polyketide synthase (PKS) capable of converting (2) into (1). A detailed protocol for making (2) is found in Example 1. General methods for making various thioesters and using the same for making polyketides are disclosed for example by U.S. Patent Nos. 6,066,721 and 6,080,555 and PCT publication WO 99/03986 which are all incorporated herein by reference. Briefly, the thioester mimics a nascent polyketide chain and thus feeds into the polyketide synthetic process starting from the first extender module of the functional PKS system.

A two-module PKS containing the appropriate domains will convert (2) into (1). The first module recognizes (2) as the starter unit, then adds a methylmalonyl extender unit and reduces the resulting β-ketone unit to an (*S*)-alcohol. The second module adds a methylmalonyl extender unit and a methyl group. Finally, a thioesterase domain produces the lactone and releases (1) from the PKS. This process is illustrated in Figure 3.

One embodiment of the invention provides a two-module fragment of a naturally-occurring PKS comprising all the above-listed activities required for conversion of (2) into (1). An example of such a fragment is modules 7 and 8 of the epothilone PKS (Figure 4), fused with a thioesterase (TE) domain so as to produce the PKS (epo)[module(7)]-[module(8)]-TE. The genes for the epothilone PKS are described in Tang *et al.*, "Recombinant methods and materials for producing epothilone and epothilone derivatives," PCT publication no. WO 00/31247 (incorporated herein by reference). The TE domain is taken either from the epothilone genes or from a heterologous gene, for example the DEBS genes. Methods for the construction and heterologous expression of two-module fragments of PKSs having fused TE domains are described in Khosla *et al.*, "Production of novel polyketides," U.S. Patent 5,712,146 (incorporated herein by reference). Compound (1) is produced according to the method of the invention when a growing culture of an organism, for example *Streptomyces coelicolor* CH999, containing an expression system for modules

7 and 8 of the epothilone PKS, is supplied with (2). The resulting (1) is extracted from the culture medium according to methods known in the art. Other heterologous expression hosts, including but not limited to *Streptomyces lividans*, *Myxococcus xanthus*, *Escherichia coli*, and *Saccharomyces cerevesiae*, may be used as described in Barr *et al.*, "Production of polyketides in bacteria and yeasts," U.S. Patents 6,033,883 and 6,258,566, and Tang *et al.*, "Recombinant methods and materials for producing epothilone and epothilone derivatives," PCT publication No. WO00/31247 (each of which is incorporated herein by reference).

Another embodiment of the invention provides a two-module PKS for the conversion of (2) into (1) resulting from the genetic engineering of a two-module fragment taken from a naturally-occurring PKS. An example of such a fragment is modules 5 and 6 of the narbonolide PKS from either *Streptomyces venezuelae* or *Streptomyces narbonensis*, along with the natural TE domain, genetically engineered so as to incorporate a methyltransferase domain in module 6 so as to produce the PKS (nar)[module(5)]-[module(6)+epoMT8]-TE (Figure 3). The genes for the narbonolide PKS and their heterologous expression have been described in McDaniel *et al.*, U.S Patent 6,117,659 (incorporated herein by reference). Suitable methyltransferase domains have also been described, for example from module 8 of the epothilone PKS. Methods for manipulating PKS domains by domain addition and replacement are described in, for example, McDaniel, "Library of Novel "Unnatural" Natural Products, PCT publication WO00/24907 (incorporated herein by reference).

Compound (1) is produced according to the method of the invention when a growing culture of an organism, for example *Streptomyces coelicolor* CH999, containing an expression system for (nar)[module(5)]-[module(6)+epoMT8]-TE, is supplied with (2). The resulting (1) is extracted from the culture medium according to methods known in the art.

As a further example, an engineered form of the DEBS3 protein of the erythromycin PKS is used. The DEBS3 protein is one of three protein subunits in the erythromycin PKS, and includes extender module 5, extender module 6, and the terminating thioesterase. Both modules naturally contain active KR domains. In this embodiment, DEBS3 is mutated so as to inactivate the ketoreductase domain of module 6

and incorporate a methyltransferase domain, so as to produce (ery)[module(5)]-[module(6)-KR°+epoMT8]-TE. In an alternate example, the KR domain is deleted, giving rise to (ery)[module(5)]-[module(6)-ΔKR+epoMT8]-TE. These PKS genes are heterologously expressed in a host such as *Streptomyces coelicolor* CH999, *Streptomyces lividans*, *Escherichia coli*, or *Saccharomyces cerevesiae.*

Two-module PKSs derived from natural PKSs other than DEBS are useful to convert (2) into (1). Modules 5 and 6 from the megalomicin PKS may also be used, for example. Recombinant methods for manipulating modular PKS genes are described, for example, by U.S. Patent Nos. 5,672,491; 5,712,146; 5,830,750; and 5,843,718; and in PCT publication Nos. 98/49315 and 97/02358, each of which is incorporated herein by reference.

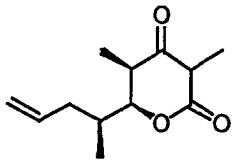
In another embodiment of the invention, a PKS for the conversion of (2) into (1) resulting from the combination of two or more modules taken from different naturally-occurring PKSs is provided. Thus, a module from DEBS and a module from the epothilone PKS may be combined. As an example, the gene (ery)[module(5)]-(epo)[module(8)]-TE is constructed and expressed in *Streptomyces coelicolor* CH999. When supplied with (2), the resulting PKS produces (1). Methods for the construction and optimization of hybrid-modules PKSs are described in Tang *et al.*, "Formation of functional heterologous complexes using subunits from the picromycin, erythromycin, and oleandomycin polyketide synthases," *Chemistry & Biology* (2000), *7*: 77:84 ; and Gokhale *et al.*, "Methods to mediate PKS module effectiveness," PCT publication WO00/47724 (both of which are incorporated herein by reference).

In yet another embodiment of the invention, a PKS containing more than two modules is provided for the conversion of Compound (2) into Compound (1), in which the first module serves as a loading module for Compound (2) and is incapable of adding an extender unit onto the polyketide chain. An example is a three-module PKS comprised of modules 1, 2, and 3 of DEBS, in which the KS of module 1 has been inactivated through, for example, mutagenesis, and a methyltransferase domain has been added to module 3, along with the terminal thioesterase domain, so as to provide (ery)[module(1)- KS°]-[module(2)]-[module(3)+epoMT8]-TE. Engineering of the three-module PKS containing

modules 1, 2, and 3 of DEBS has been described in McDaniel *et al.*, "Gain-of-function mutagenesis of a modular polyketide synthase," *J. Am. Chem. Soc.* (1997) *119*: 4309-4310, (incorporated herein by reference). Construction of the [KS°] mutant is done either by mutagenesis of the three-module construct to introduce the [KS°] mutation, or by addition of module 3 into the existing two-module [KS°] mutant described in Khosla *et al.*, "Synthesis of Polyketides from diketides," U.S. Patent 6,080,555 (incorporated herein by reference).

While illustrated using PKS modules derived from the erythromycin PKS, DEBS, other sources of modules may be used in accord with the methods of the invention, including but not limited to the PKSs involved in the biosythesis of erythromycin, megalomicin, picromycin, narbomycin, oleandomycin, lankamycin, FK506, FK520 (ascomycin), rapamycin, epothilone, tylosin, spiramycin, rosamicin, geldanamycin, pimaricin, FR008, candicidin, avermectin, and the like.

In another aspect of the invention, compound (3) of formula



(3)

is prepared using an engineered polyketide synthase. This compound (3) is converted according to the methods of the invention through a short series of chemical transformations into (1), and thus serves as a precursor for the synthesis of polyketides such as epothilone. The stereochemistry of the C2-methyl group in (3) is unspecified, due to the ease of epimerization of this center and the utility of both diastereomers in the synthesis of epothilones.

The production of (3) according to the methods of the invention differs from the above-described production of (1) in that a methyltransferase domain is not used to add the second C2-methyl group in (1). A PKS comprising one module having a methylmalonyl-specific AT domain and *(S)*-specific KR domain and a second module having a methylmalonyl-specific AT domain and a thioesterase is provided by the invention for the conversion of Compound (2) into Compound (3).

One embodiment of the invention provides a two-module fragment of a naturally-occurring PKS comprising all the above-listed activities required for conversion of Compound (2) into Compound (3). An example of such a fragment is modules 5 and 6 of the narbonolide PKS from either *Streptomyces venezuelae* or *Streptomyces narbonensis*, (nar)[module(5)]-[module(6)]-TE. Compound (3) is produced according to the method of the invention when a growing culture of an organism, for example *Streptomyces coelicolor* CH999, containing an expression system for (nar)[module(5)]-[module(6)]-TE, is supplied with (2). The resulting (3) is extracted from the culture medium according to methods known in the art. Other heterologous expression hosts, including but not limited to *Streptomyces lividans*, *Myxococcus xanthus, Escherichia coli,* and *Saccharomyces cerevesiae*, may be used.

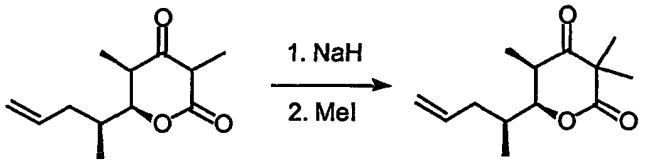
Another embodiment of the invention provides a two-module PKS for the conversion of (2) into (3) resulting from the genetic engineering of a two-module fragment taken from a naturally-occurring PKS. As an example, an engineered form of the DEBS3 protein of the erythromycin PKS is used, (ery)[module(5)]-[module(6)-KR°]-TE or (ery)[module(5)]-[module(6)-ΔKR]-TE. Two-module PKSs derived from natural PKSs other than DEBS are useful to convert (2) into (3). Modules 5 and 6 from the megalomicin PKS may also be used, for example.

In another embodiment of the invention, a PKS for the conversion of (2) into (3) resulting from the combination of two or more modules taken from different naturally-occurring PKSs is provided. Thus, a module from DEBS and a module from the narbonolide PKS may be combined, as in (ery)[module(5)]-(nar)[module(6)]-TE.

In yet another embodiment of the invention, a PKS containing more than two modules is provided for the conversion of (2) into (3), in which the first module serves as a loading module for (2) and is incapable of adding an extender unit onto the polyketide chain. An example is a three-module PKS comprised of modules 1, 2, and 3 of DEBS, in which the KS of module 1 has been inactivated through, for example, mutagenesis, along with the terminal thioesterase domain so as to provide (ery)[module(1)- KS°]-[module(2)]-[module(3)]-TE. Modules from several PKS genes can also be used, for example (ery)[module(1)- KS°]-[module(2)]-(nar)[module(6)]-TE.

While illustrated using PKS modules derived from the erythromycin PKS, DEBS, other sources of modules may be used in accord with the methods of the invention, including but not limited to the PKSs involved in the biosythesis of erythromycin, megalomicin, picromycin, narbomycin, oleandomycin, lankamycin, FK506, FK520 (ascomycin), rapamycin, epothilone, tylosin, spiramycin, rosamicin, geldanamycin, pimaricin, FR008, candicidin, avermectin, and the like.

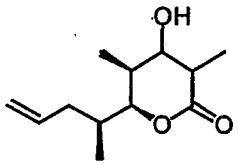
Compound (3) is converted into (1) according to the methods of the invention by chemical methylation. Treatment of (3) with a base, for example sodium hydride, potassium *tert*-butoxide, and the like, followed by treatment with a methylation reagent, for example methyl iodide, converts (3) into (1).



(3) (1)

An illustrative protocol for a chemical methylation reaction is found in Example 7.

In another aspect of the invention, a compound of formula (4) is provided:



(4)

differing from (3) in having a C3-alcohol rather than a C3-ketone. According to the methods of the invention, (4) is converted into (3) by oxidation. The stereochemistry of the C3-alcohol is not specified, as both isomers yield (3) upon oxidation and so are useful according to the methods of the invention. The stereochemistry of the C2-methyl is not specified, as both isomers yield (1) upon oxidation and subsequent methylation, and so are useful according to the methods of the invention.

The production of (4) from (2) according to the methods of the invention differs from the above-described production of (3) in that a functional KR domain in the second module provides the C3-alcohol. A PKS comprising one module having a methylmalonyl-

specific AT domain and *(S)*-specific KR domain and a second module having a methylmalonyl-specific AT domain, an active KR, and a thioesterase is provided by the invention for the conversion of **(2)** into **(4)**.

One embodiment of the invention provides a two-module fragment of a naturally-occurring PKS comprising all the above-listed activities required for conversion of **(2)** into **(4)**. As an example, the DEBS3 protein of the erythromycin PKS is used, (ery)[module(5)]-[module(6)]-TE. In this embodiment, DEBS3 is heterologously expressed in a host such as *Streptomyces coelicolor* CH999, *Streptomyces lividans*, *Escherichia coli*, or *Saccharomyces cerevesiae*. Two-module PKSs derived from natural PKSs other than DEBS are useful to convert **(2)** into **(4)**, including but not limited to the PKSs involved in the biosythesis of erythromycin, megalomicin, picromycin, narbomycin, oleandomycin, lankamycin, FK506, FK520 (ascomycin), rapamycin, epothilone, tylosin, spiramycin, rosamicin, geldanamycin, pimaricin, FR008, candicidin, avermectin, and the like.

In another embodiment of the invention, a PKS for the conversion of **(2)** into **(4)** resulting from the combination of two or more modules taken from different naturally-occurring PKSs is provided. Thus, a module from DEBS and a module from the narbonolide PKS may be combined, for example, as in (nar)[module(5)]-(ery)[module(6)]-TE.

In yet another embodiment of the invention, a PKS containing more than two modules is provided for the conversion of **(2)** into **(4)**, in which the first module serves as a loading module for **(2)** and is incapable of adding an extender unit onto the polyketide chain. An example is a three-module PKS comprised of modules 1, 5, and 6 of DEBS, in which the KS of module 1 has been inactivated through, for example, mutagenesis, along with the terminal thioesterase domain so as to provide (ery)[module(1)- KS^{o}]-[module(5)]-[module(6)]-TE.

While illustrated using PKS modules derived from the erythromycin PKS, DEBS, other sources of modules may be used in accord with the methods of the invention, including but not limited to the PKSs involved in the biosythesis of erythromycin, megalomicin, picromycin, narbomycin, oleandomycin, lankamycin, FK506, FK520

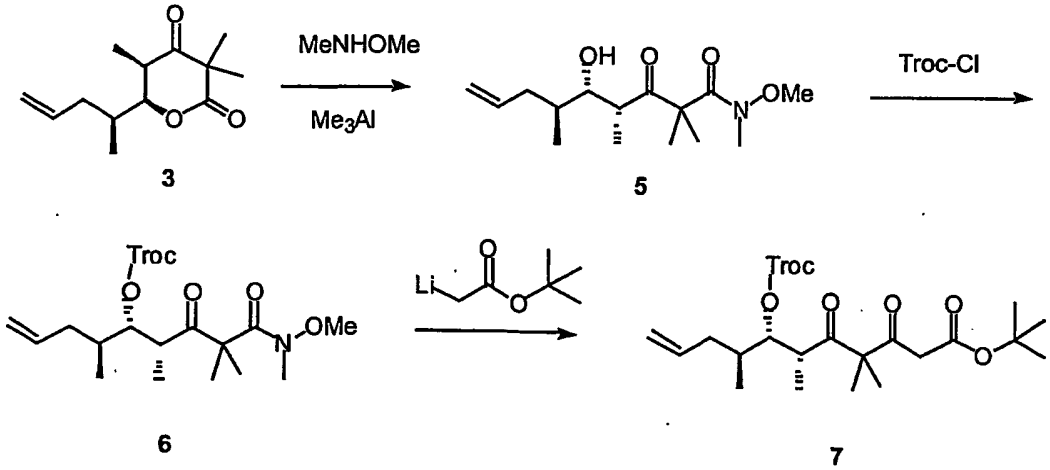
(ascomycin), rapamycin, epothilone, tylosin, spiramycin, rosamicin, geldanamycin, pimaricin, FR008, candicidin, avermectin, and the like.

Although additional chemical modifications are necessary with some of these embodiments, the methods of the invention nevertheless provide the following advantages. First, the methods are relatively inexpensive. Due to the inherent stereoselectivity of biological systems, a racemic mixture of the SNAc compound may be used instead of as a pure enantiomer. Second, the expression of DEBS and other PKS genes in *Streptomyces coelicolor* is well characterized and the hydroxy lactone product may be made in relatively large quantities by simple fermentations.

The hydroxyl group of (4), which will become the future C-5 keto group of epothilone, may be chemically oxidized according to the methods of the invention with a mild oxidizing agent such as methylsufoxide/oxalyl chloride/triethylamine (i.e., Swern oxidation), methylsulfoxide/carbodiimide (i.e., Moffat oxidation), chromic acid (H_2CrO_4), hypervalent iodine oxidants (e.g., IBX, Dess-martin periodinane), and the like to provide (3). An illustrative example of such an oxidative protocol is found in Example 6.

In a further aspect of the invention, methods for the conversion of (3) into Compound A are provided. Scheme 1 illustrates one embodiment of the invention.

SCHEME 1



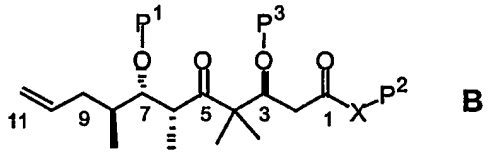
3 5 6 7

Briefly, the lactone of (3) is opened using N,O-dimethylhydroxylamine and trimethylaluminum to form the Weinreb amide, (5), and the resulting free hydroxyl group

is protected using trichloroethyl chloroformate ("Troc-Cl") to provide (6). The Weinreb amide is reacted with a source of nucleophilic acetate, for example the lithium enolate of *tert*-butyl acetate to yield (7), Compound A wherein P^1 is 2,2,2-trichloroethoxycarbonyl ("Troc"), P^2 is *tert*-butyl, and X is oxygen. Substitution of trichloroethyl chloroformate by another protecting reagent, including but not limited to trisubstituted silyl chlorides, trisubstituted silyl trifluoromethanesulfonates ("triflates"), and the like, gives rise to Compound A wherein P^1 is the corresponding protecting group, for example a trialkylsilyl ether. Particularly preferred examples include P^1 = Troc, *tert*-butyldimethylsilyl ("TBS"), and triethylsilyl ("TES").

In a second embodiment of the invention, the *tert*-butyl acetate of Scheme 1 is replaced with *tert*-butyl thioacetate, resulting in the formation of Compound A wherein P^1 is Troc, TBS, or TES, P^2 is *tert*-butyl, and X is sulfur.

In another aspect of the present invention, Compound B is provided that already includes the future C-3 alcohol with the appropriate stereochemistry:

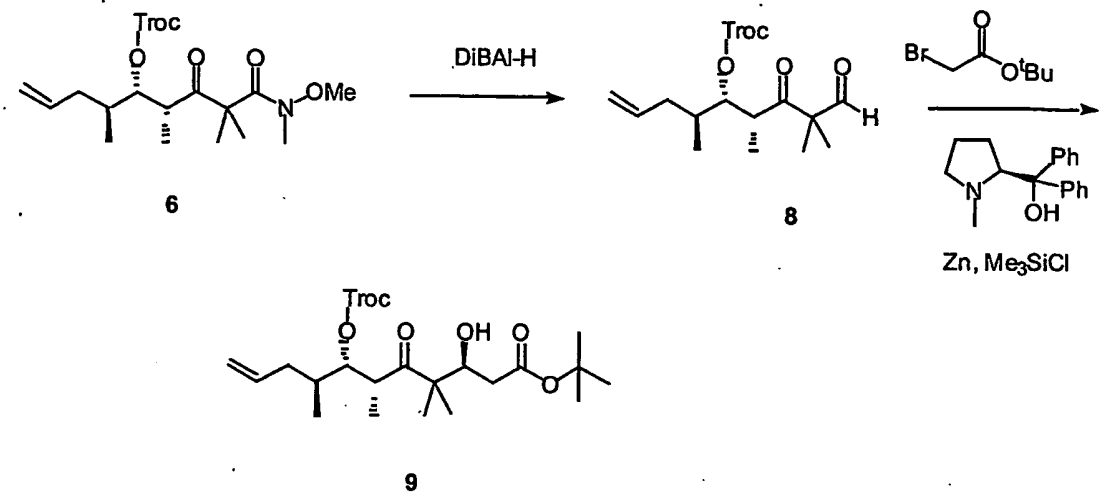


B

Wherein P^1, P^2, and P^3 are H or protecting groups and X is oxygen or sulfur.

One embodiment for making Compound B from (6), and hence from (3), according to the methods of the present invention is illustrated by Scheme 2.

SCHEME 2



6 8 9

As described above, (1) is converted into (6). Di-isobutyl aluminum hydride is used to reduce the amide to aldehyde (8). An aldol or similar reaction subsequently stereoselectively extends the aldehyde by two carbons and sets the stereochemistry of the 3-alcohol. In one embodiment, an asymmetric Reformatsky reaction is performed using *tert*-butyl bromoacetate and zinc in the present of a chiral proline-derived ligand. In this embodiment, the product is (9), Compound B, wherein P^1 = Troc, P^2 = *tert*-butyl, P^3 = H, and X= O. This is reacted with a hydroxyl protecting reagent, for example triethylchlorosilane, to protect the 3-OH to give (10), compound B wherein P^1 = Troc, P^2 = *tert*-butyl, P^3 = Et_3Si ("TES"), and X= O. Use of reagents other than triethylchlorosilane can be used to introduce other P^3 protecting groups. Preferred examples of P^3 protecting groups include TES and TBS.

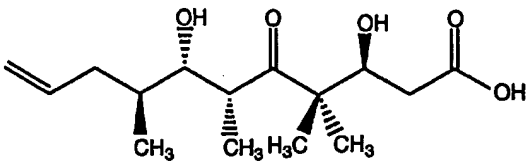
According to the methods of the present invention (Figure 6), (10) and the Danishefsky thiazole intermediate are coupled via the Suzuki coupling method, lactonized, and deprotected to yield epothilone D.

In another aspect of the present invention, Compound B, wherein P^1 = H, P^2 = H, P^3 = H, and X= O (11), is made directly using biological methods. The method generally involves the use of a library of polyketides where the library includes information regarding the structures of the polyketides. In preferred embodiments, the polyketide structures in the library are represented in linear form. The linear form may be a linearized version of the chemical structure. The linearized polyketide structures are a convenient

representation for comparing one polyketide structure with another. In more preferred embodiments, each linearized polyketide structure is divided into a sequence of two carbon units. In even more preferred embodiments, the chemical structures of the two-carbon units are represented by symbols such that the structural sequence of two carbon units are now transformed into a linear sequence of symbols. The linearized structures are further decomposed into two-carbon units which in turn are each represented by a symbol. A general method for assigning symbols to structural fragments is described in greater detail in U.S. Patent Application 01/17352. Briefly, a modified version of the CHUCKLES methodology is provided to represent polyketide structures (see Siani *et al.*, CHUCKLES: a method for representing and searching peptide and peptoid sequence, *J. Chem. Inf. Comp. Sci*, 1994 34: 588-593 which is incorporated herein by reference).

The precise method for designing a gene for making a polyketide compound such as (11) depends on how the library of polyketides is organized. In general, the method comprises comparing the structure of the compound to be synthesized with the structures of the polyketides in the library and using this information to design a gene that when expressed in cells will make a PKS enzyme that can be used to synthesize the desired compound.

In one embodiment, (11), having the formula



(11)

is made by using a subset of the epothilone PKS gene since epothilone itself includes the most number of consecutive matching two-carbon units with the target sequence (Compound 11) of two-carbon units. As a result, a variation of the inventive method is used. The general method comprises:

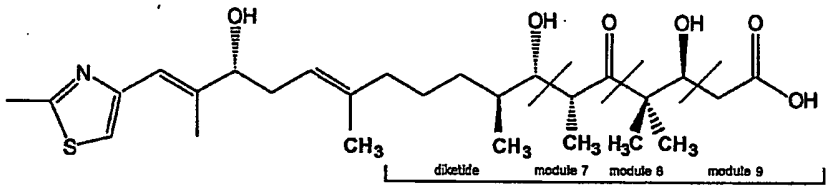
describing at least a portion of the target compound as a sequence of two-carbon units;

associating a PKS gene that results in the production of the naturally occurring polyketide;

determining the fragment of a PKS gene that is associated with making the compound's sequence of two-carbon units and;

designing a new gene that includes the fragment of the PKS gene.

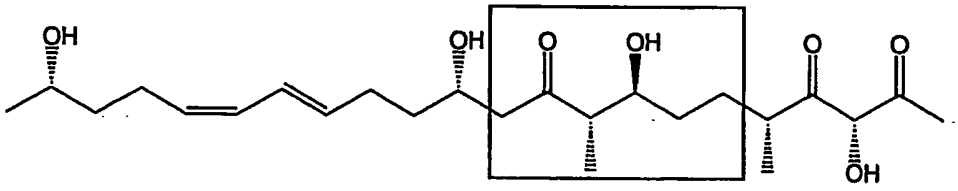
The epothilone PKS gene has been cloned as described by PCT publication No. WO00/31247, which is incorporated herein by reference. As it can be seen from comparing the structure of (11) to a linearized representation of the epothilone structure



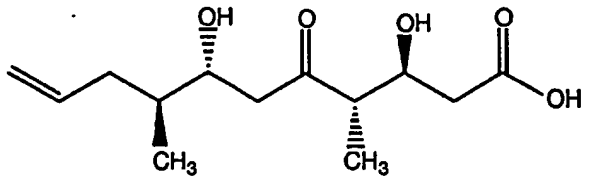
,

Compound (11) (not surprisingly) is virtually identical to the right hand portion of the epothilone structure. In addition, the right hand portion may be divided into four two-carbon units. Thus in one method, (2) is added to a PKS system comprising a functional EpoE and EpoF subunits, (epo)[module(7)]-[module(8)]-[module(9)]-TE. The functional PKS system may be in a suitable host cell or may be part of a cell free system.

A number of alternate methods of the present invention can be used to make (11) (or any other polyketide. When (11) is made using non-epothilone PKS genes, practice of the present invention is as described previously. For example, in another embodiment, a subset of modules from tartralone B ("tar") is used to make (11) A linearized representation of tartralone B is as follows:



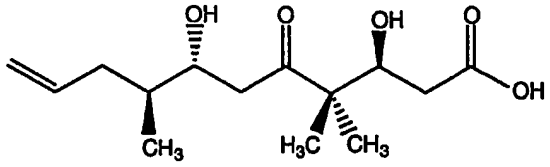
where the boxed portion of the structure is due to extender modules 5 through 7 of the tartralone B PKS enzyme. As a result, a compound (12) similar to (11) having the formula



(12)

may be made by adding (2) to a PKS system comprising (tar)[module(5)]-[module(6)]-[module(7)]-TE. Compound (11) can be made by modifing the PKS system by replacing the AT in tartralone B extender module 5 to an AT specific for methyl malonyl CoA (e.g. the AT in tartralone B extender module 6) and inserting the methyl transferase domain from epothilone extender module 8 into tartralone B extender module 6, thus producing (tar)[module(5)-AT/tarAT6]-[module(6)+epoMT8]-[module(7)]-TE.

In yet another embodiment, a similar method is used to make (11) except that borophycin ("bor") extender modules 4 through 6 are used instead of the tartralone B extender modules. If Compound (2) is added to a host cell expressing (bor)[module(4)]-[module(5)]-[module(6)]-TE, then the following polyketide (13) would result:



. (13).

Compound (11) is made by modifying the AT in the borophycin extender module 4 to one specific for methyl malonyl CoA, for example in (bor)[module(4)-AT/tarAT6]-[module(5)]-[module(6)]-TE.

Similarly, modified portions of the aplasmomycin ("apl") and boromycin PKS genes may be used to make (11). Illustrative examples of modified gene constructs are:

(apl)[module(3)-AT/tarAT6-DH°]-[module(4)]-[module(5)]-TE

(bor)[module(3)-AT/rapAT2-DH°]-[module(4)]-[module(5)]-TE

While the boromycin module 3 contains the complete set of DH/ER/KR modification domains, inactivation of the DH domain is sufficient to insure inactivation of the ER domain, as DH activity is a prerequisite for ER activity.

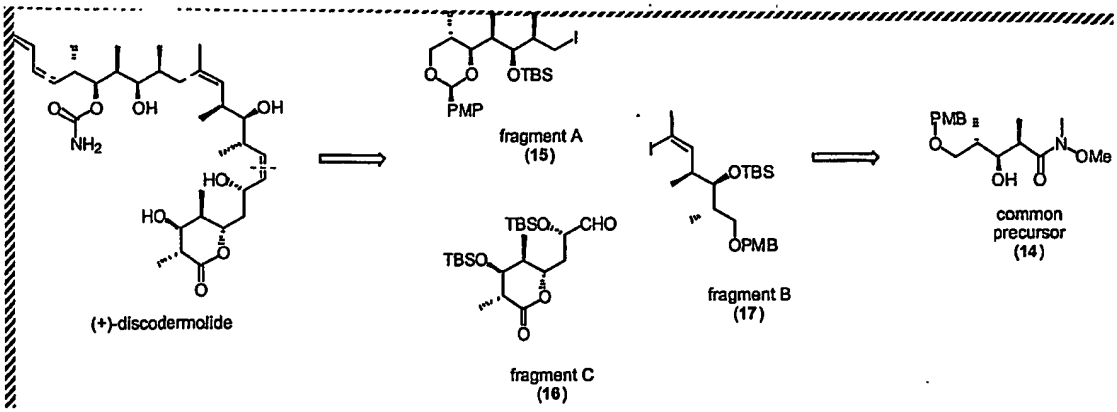
In another embodiment, the previously described (1) is used in another novel protocol to yield epothilone D. As illustrated by Figure 7, the methylated keto-lactone is coupled to the thiazole intermediate using Suzuki coupling method. The extended lactone is opened to the Weinreb amide and the resulting free hydroxyl is protected. The amide is reduced to an aldehyde and is extended by two carbons. The resulting product is reacted with Bu_4NF which removes the protecting groups and forms the lactone at the same time to yield epothilone D.

Discodermolide

In another aspect of the present invention, a combination of biological and chemical methods is provided for making discodermolide analogs and intermediates useful in the synthesis of discodermolide analogs. Initial studies of discodermolide in tubulin polymerization assays suggest it possesses potent anti-cancer properties. As with epothilone, more extensive investigations are hampered by the small quantities of discodermolide that can be obtained from naturally occurring sources.

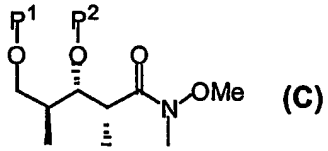
In an effort to circumvent this problem, several research groups have succeeded in completing the *de novo* chemical syntheses of discodermolide. Of these, the synthesis reported by Smith and co-workers (Scheme 3) is particularly notable because it uses a modular approach where three fragments that are derived from a common precursor, (14), are joined together. See e.g., Smith *et al.*, 1999, Gram-scale synthesis of (+)-discodermolide, *Org Lett.* 1(11):1823-6; U.S. Patent Nos. 6,096,904, 6,031,133, and, 5,789,605; and PCT Publication Nos. WO 00/04865 and WO 98/24429, each of which is incorporated herein by reference.

SCHEME 3



The Smith synthesis is long and complex, and generally not amenable for making commercial quantities. Because discodermolide is not the only polyketide whose use is hampered by inadequate supply, a need exists for novel approaches for obtaining these important compounds.

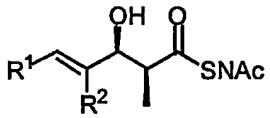
In one embodiment of the present invention, Smith's common precursor (14) is made using a combination of biological and chemical methods. Smith's common precursor is a compound of formula (C)



(C)

wherein P^1 is 4-methoxybenzyl ("PMB") and P^2 is H.

The method of the invention comprises adding a compound of formula (D):



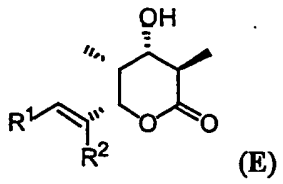
(D)

wherein

R^1 is H, alkyl, or aryl; and

R^2 is H or alkyl;

to a functional PKS system to make a lactone (E) of the following formula



(E)

wherein

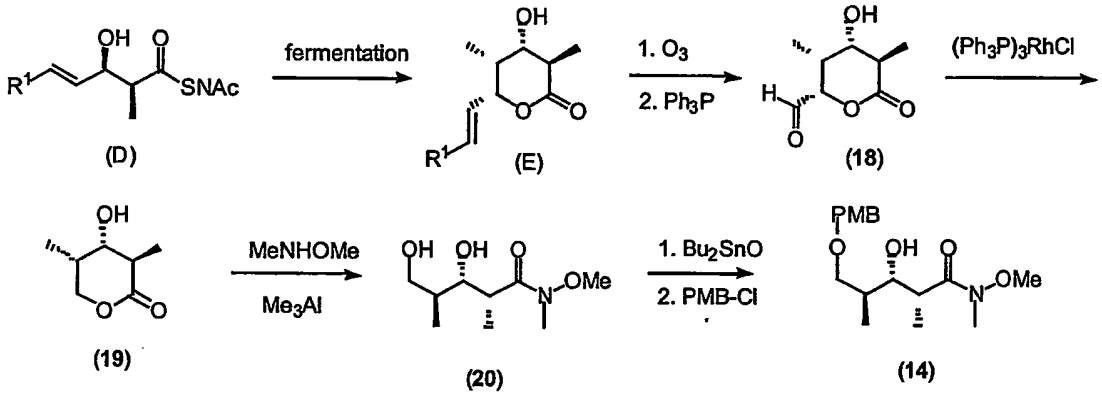
R^1 is H, alkyl, or aryl; and

R^2 is H or alkyl.

Preferred examples of suitable functional PKSs include (ery)[module(1)-KS°]-[module(2)]-TE, (ole)[module(1)-KS°]-[module(2)]-TE, and (meg)[module(1)-KS°]-[module(2)]-TE.

Scheme 4 illustrates one embodiment of the invention, wherein R^2 is H, where the lactone (E) resulting from feeding Compound (D) to the (ery)[module(1)-KS°]-[module(2)]-TE polyketide synthase is chemically modified to yield Smith's chiral precursor (14).

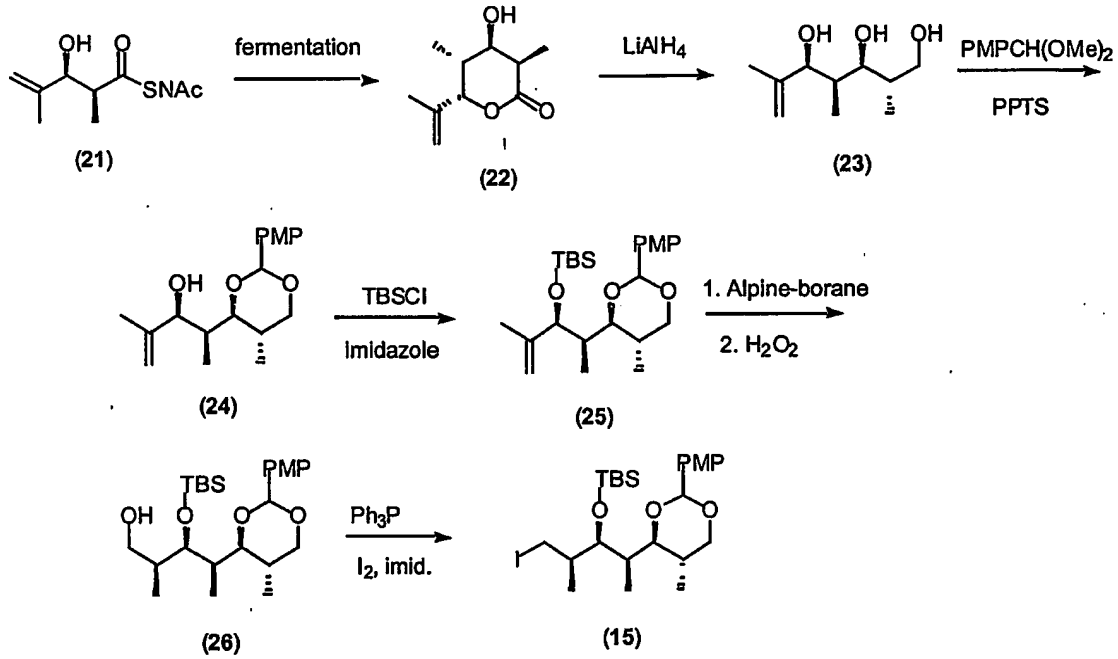
SCHEME 4



The vinyl group of the lactone is oxidized to the aldehyde (18), for example using ozonolysis with a reductive workup or using a two-step process of osmium tetraoxide followed by sodium periodate. The resulting aldehyde is decarbonylated to yield (19), for example using Wilkinson's catalyst (tris(triphenylphosphine)rhodium chloride). Lactone (19) can be converted into (14) by conversion to the Weinreb amide (20) followed by selective protection of the terminal primary alcohol. In a preferred example, this protection is performed by initial formation of the cyclic dibutylstannoxane, followed by reaction with an alkylating agent such as 4-methoxybenzylchloride.

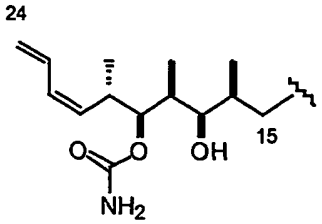
In another embodiment of the invention, the lactone (22) resulting from feeding compound (D) wherein R^1 is H and R^2 is methyl (21) to a host cell expressing the (ery)[module(1)-KS°]-[module(2)-KR/rapKR2]-TE PKS or equivalent is used as shown in Scheme 5 to prepare Smith's "fragment A" of discodermolide (15).

SCHEME 5

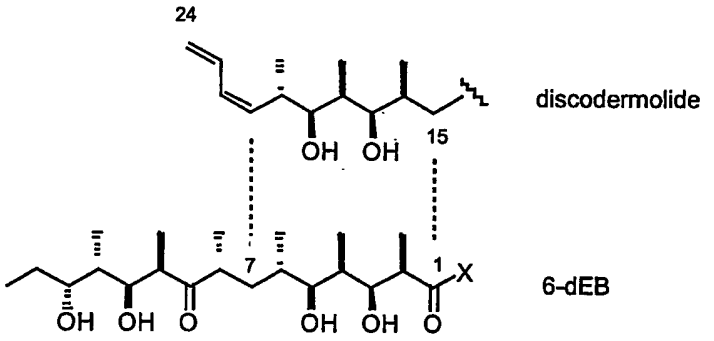


Replacement of the KR2 in DEBS1+TE with the module 2 KR from the rapamycin PKS results in formation of the lactone (22) having the opposite stereochemistry at the 3-alcohol. Lactone (22) is reduced, for example with lithium aluminum hydride, and the resulting triol (23) is protected by sequential treatment with (4-methoxyphenyl)benzaldehyde dimethyl acetal ($PMPCH(OMe)_2$) under acid catalysis, such as pyridinium *para*-toluenesulfonate (PPTS) followed by *tert*-butyldimethylsilyl chloride and base to yield (25). The alkene is stereoselectively hydroborated using Alpine-borane (*B*-isopinocampheyl-9-borabicyclo[3.3.1]nonane) with an oxidative workup to provide the primary alcohol (26). Conversion of the alcohol to the primary iodide using iodine and triphenylphosphine in the present of imidazole provides (15).

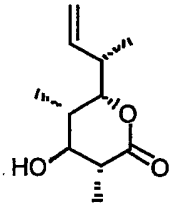
In another embodiment, genetically-engineered polyketide synthases are provided which yield compounds useful in the preparation of the C15-C24 segment of discodermolide more directly:



Comparison with the saw-tooth representation of 6-dEB reveals that this stereochemically complex segment of discodermolide may be produced by the protein that produces the C1-C7 fragment of 6-dEB:

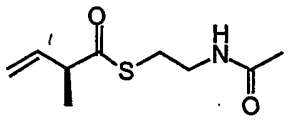


In one embodiment of the invention, a PKS is constructed and used to produce a compound (27) of the formula



(27)

which serves as a precursor for synthesis of the C15-C24 segment of discodermolide. Examination of the sawtooth projection of (27) reveals that a suitable PKS would contain two modules equivalent in function to DEBS modules 5 and 6, found in the DEBS3 protein, along with the TE domain. Thus, in this embodiment, heterologously expressed (ery)[module(5)]-[module(6)]-TE is supplied with a thioester of the formula (28)

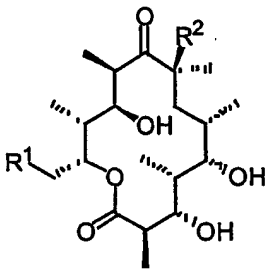


(28)

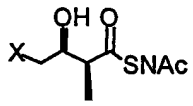
in order to produce (27). Again, while illustrated using modules taken from the DEBS gene cluster, other modules containing equivalent domains are suitable for the method of the invention.

In a second embodiment, a three-module construct (ery)[module(1)-KS°]-[module(5)]-[module(6)]-TE is constructed. Supplying a host cell expressing this PKS with (**28**) also results in the formation of (**27**).

In another aspect of the invention, 6-dEB analogs of formula (**F**) useful in the synthesis of discodermolide and other polyketides are provided:



wherein R^1 is halogen or phenylthio; and R^2 is H or OH. The compounds are prepared by first contacting a PKS, for example the complete erythromycin PKS containing the KS1° mutation, with a thioester of formula (**G**)



(**G**)

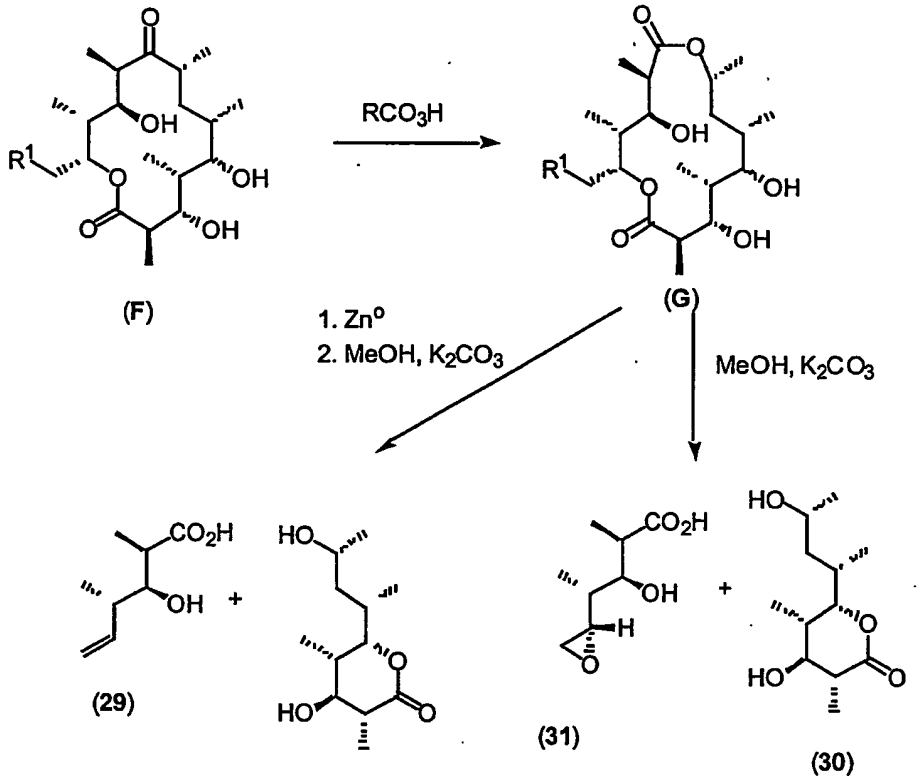
so as to produce compounds of formula (**F**) wherein R^2 = H, then optionally contacting said product with a cytochrome P450-type hydroxylase specific for hydroxylation at C8 to produce compounds of formula (**F**) wherein R^2 = OH.

Such hydroxylases are known from natural gene clusters, for example the oleandomycin and lankamycin gene clusters from *Streptomyces antibioticus* and *Streptomyces violaceoniger*, respectively. In oleandomycin, the *oleP* gene encodes a cytochrome P450 hydroxylase which adds a hydroxy group to the 8-position of 6-dEB, as described in McDaniel et al., "Production of 8,81-dihydroxy-6-deoxyerythronolide B," U.S. Patent Application 09/768,927 (incorporated herein by reference). In lankamycin, the *lkm* P450 gene encodes an 8-hydroxylase.

The use of racemic precursors (**G**) to produce 6-dEB analogs (**F**) wherein R^2 = H is described in Ashley *et al.*, "Synthesis of oligoketides," PCT application no.US00/02397 (incorporated herein by reference).

In one embodiment of the invention, analogs (F) wherein $R^2 = H$ are converted into intermediates useful in the synthesis of discodermolide, illustrated in Scheme 6.

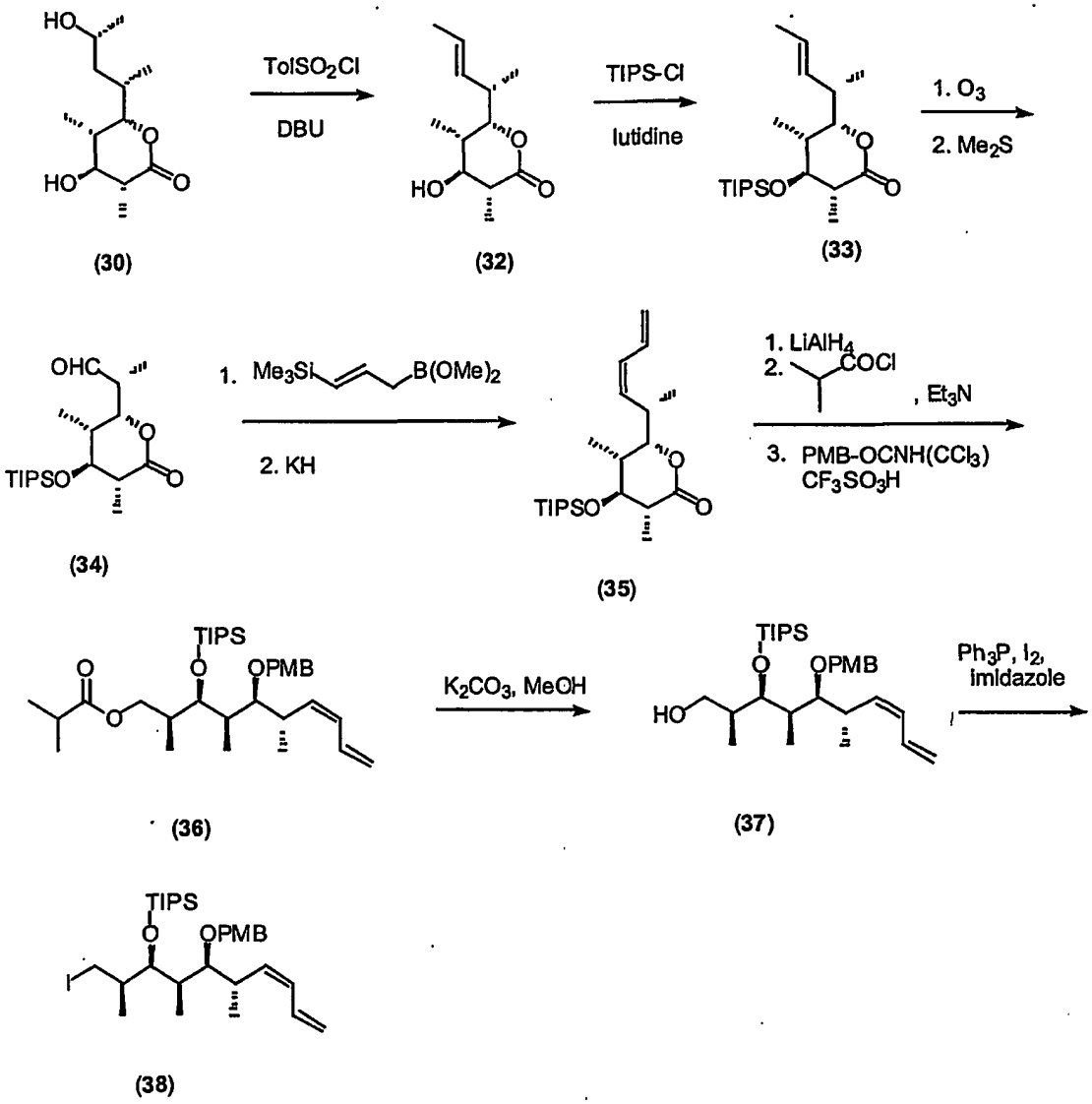
SCHEME 6



Bayer-Villager oxidation of the C9-ketone of (F) using a peracid, for example trifluoroperacetic acid, peracetic acid, *m*-chloroperbenzoic acid, monopermaleic acid, and the like, yields the ring-expanded dilactone (G). When R^1 = SPh, the peracid treatment also oxidizes the thioether into the sulfone SO_2Ph. In one embodiment of the invention, (G) is treated with zinc followed by methanolic base, generated for example by the use of potassium carbonate in methanol, to cleave the molecule into two fragments, acid (29) and lactone (30). In a second embodiment of the invention, the dilactone is treated with methanolic base directly to generate fragments (30) and (31).

Lactone (30) contains the stereogenic centers present in the C15-C24 fragment of discodermolide, and in an embodiment of the invention (Scheme 7) is converted into a fragment suitable for the synthesis of discodermolide and analogs.

SCHEME 7



Elimination of the C8-alcohol, for example by activation through conversion to a sulfonate ester (tosylate, mesylate, triflate, and the like) followed by treatment with a base (for example, 1,8-diazabicyclo[5.4.0]undec-7-ene, "DBU") yields the 7,8-alkene (**32**). The 3-hydroxyl group is protected, for example using a trialkylsilyl ether (**33**). Preferred protecting groups include triisopropylsilyl (TIPS), TES, and TBS. Ozonolysis of the alkene to the aldehyde is followed by installation of the discodermolide diene segment to yield intermediate (**34**). Several methods for construction of the diene (**35**) from the aldehyde are known, including the use of trimethylsilylallylboronates, Harried *et al*, "Total synthesis of (-)discodermolide: An application of a chelation-controlled alkylation reaction," *J. Org. Chem.* (1997) *62*: 6098-6099; α-(trimethylsilyl)allyl bromide, Marshall *et*

al., "Synthesis of discodermolide subunits by S_E2' addition of nonracemic allenylstannanes to aldehydes," *J. Org. Chem.* (1998) *63*: 817-823; and phosphinoallyl titanium reagents, Smith *et al.*, "Gram-scale synthesis of (+)-discodermolide," *Organic Letters* (1999), *1*: 1823-1826 (each of which is incorporated herein by reference). The preferred method for introducing the diene to form (XV) is the addition of a 3-(trialkylsilyl)allylboronate, particularly dimethyl 3-(trimethylsilyl)allylboronate, to the aldehyde, followed by treatment with a strong base, particularly KH.

Elaboration of intermediate (**35**) into discodermolide intermediate (**38**) according to the present invention involves reduction of the lactone carbonyl to a primary alcohol followed by differential protection of the resulting hydroxyl groups and final conversion of the primary alcohol into a group suitable for coupling to a second discodermolide fragment.

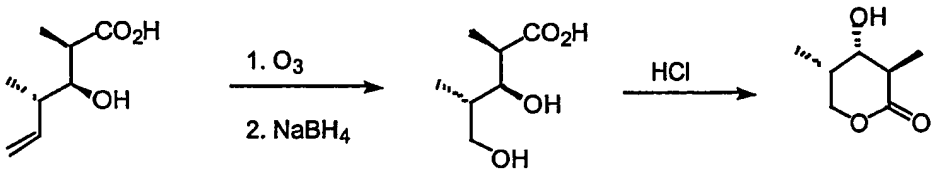
In one embodiment, (**35**) is first reduced using a hydride reagent. Preferred examples of such hydride reagents include lithium aluminum hydride, lithium triethylborohydride, and sodium borohydride. The resulting primary alcohol is selective protected. Preferred examples of protecting groups include the isobutyrate ester, acetate ester, benzoate ester, triphenylmethyl ether, and di(4-methoxyphenyl)phenylmethyl ether. The isobutyrate ester is particularly preferred. The remaining secondary alcohol is then protected as a p-methoxybenzyl (PMB) ether, introduced either by reaction with p-methoxybenzyl trichloroacetimidate under acid catalysis or by reaction with p-methoxybenzyl bromide under base catalysis. Preferred examples of acid catalysts include trifluoromethanesulfonic acid and pyridinium p-toluenesulfonate (PPTS). Preferred examples of base catalysts include pyridine, diisopropylethylamine, and sodium hydride. After introduction of the PMB ether, the primary alcohol is deprotected to yield (**37**). When the protecting group is an ester, deprotection uses a mixture of potassium carbonate in methanol. When the protecting group is a trityl or DMT ether, deprotection uses chlorocatechol borane in methanol.

According to the methods of the invention, the liberated primary alcohol is converted into the iodide using triphenylphosphine and iodine in the presence of a base such as imidazole, yielding discodermolide fragment (**38**). This fragment can be incorporated into discodermolide or discodermolide analogs using methods known in the

art, for example Marshall and Johns, "Total synthesis of (+)-discodermolide," *J. Org. Chem.* (1998), *63*: 7885-7892, incorporated herein by reference.

In another embodiment of the invention, fragment (**29**) is used to prepare the Smith "chiral precursor" (**14**). As shown in Scheme 8, the alkene of (XI) is oxidized to an aldehyde. Preferred methods for oxidation include ozonolysis and osmium tetraoxide/sodium periodate; ozonolysis is particularly preferred. Reduction of the aldehyde to the alcohol (**39**), preferably by treatment of the intermediate ozonide with sodium borohydride, followed by acid-catalyzed lactonization provides compound (**19**), which is converted into the Smith precursor as described above.

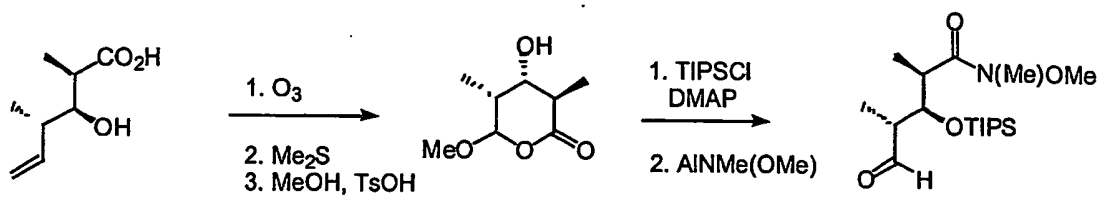
SCHEME 8



(**29**) (**39**) (**19**)

In another embodiment, (**29**) is used to prepare another intermediate useful in the synthesis of discodermolide and its analogs. As shown in Scheme 9, the alkene of (**29**) is oxidized to an aldehyde. Preferred methods for oxidation include ozonolysis and osmium tetraoxide/sodium periodate; ozonolysis is particularly preferred. The aldehyde is trapped as a methyl lactone acetal (**40**) by treatment with acidic methanol. The free hydroxyl group is protected. Preferred examples of protecting groups include trialkylsilanes such as TMS, TES, TBS, and TIPS; TIPS is particularly preferred. The lactone is opened to the Weinreb amide (**41**) by treatment with N,O-dimethylhydroxylamine. Compound (**41**) is used identically with compound "(-)-8" in Smith *et al.*, "Gram-scale synthesis of (+)-discodermolide," *Organic Letters* (1999) *1*: 1823-1826, to produce discodermolide, differing only in the substitution of a TIPS protecting group for a TBS protecting group.

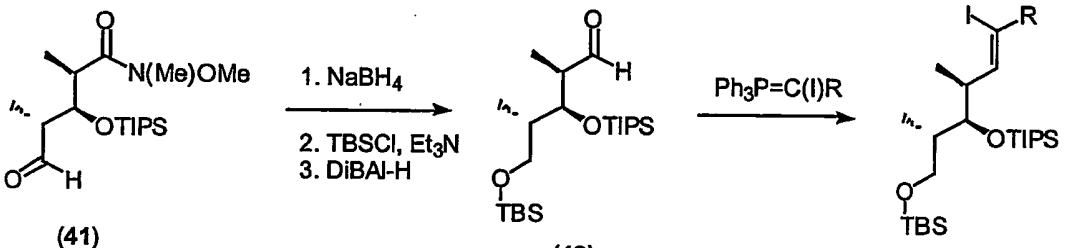
SCHEME 9



(29) (40) (41)

In another embodiment, (41) is used according to the methods of the invention as a precursor to the C9-C14 segment of discodermolide (Scheme 10). Reduction of the aldehyde using sodium borohydride is followed by protection of the alcohol with TBS to yield. Reduction of the Weinreb amide using diisobutylaluminum hydride (DiBAl-H) yields an aldehyde (42) which is converted into the vinyl iodide (43) using a Wittig reagent. Preferred examples of Wittig reagents include (iodomethylidene)triphenylphosphorane, (1-iodoethylidene)-triphenylphosphorane, and the like.

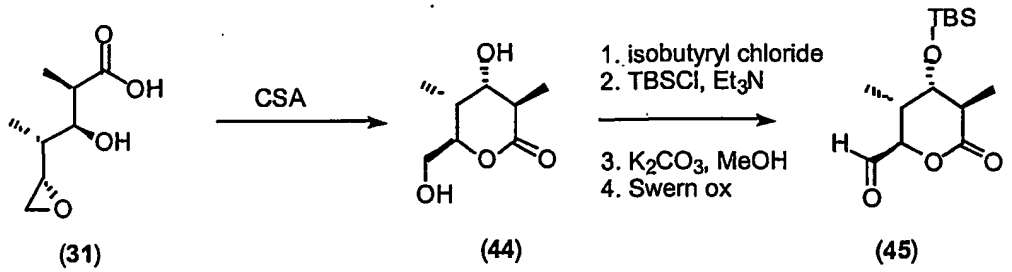
SCHEME 10



(41) (42) (43)

In another embodiment of the invention, the fragment (31) is used to prepare the C1-C8 segment of discodermolide. As shown in Scheme 11, fragment (31) is lactonized by treatment with an acid catalyst. Preferred examples of acid catalysts include 10-camphorsulfonic acid ("CSA"), toluenesulfonic acid, methanesulfonic acid, and the like. The resulting lactone (44) is differentially protected at the 6-alcohol, preferably using an ester, most preferably using an isobutyrate ester, and at the 3-alcohol, preferably using a trialkylsilyl ether, and most preferably using a TBS ether. The 6-alcohol is then deprotected using potassium carbonate in methanol, and then oxidized to an aldehyde (45). The oxidation is preferably performed using methylsulfoxide/oxalyl chloride/triethylamine (i.e., Swern oxidation), a hypervalent iodine reagent (Dess-martin periodinane or IBX), or chromium trioxide in pyridine, most preferably using methylsulfoxide/oxalyl chloride/triethylamine.

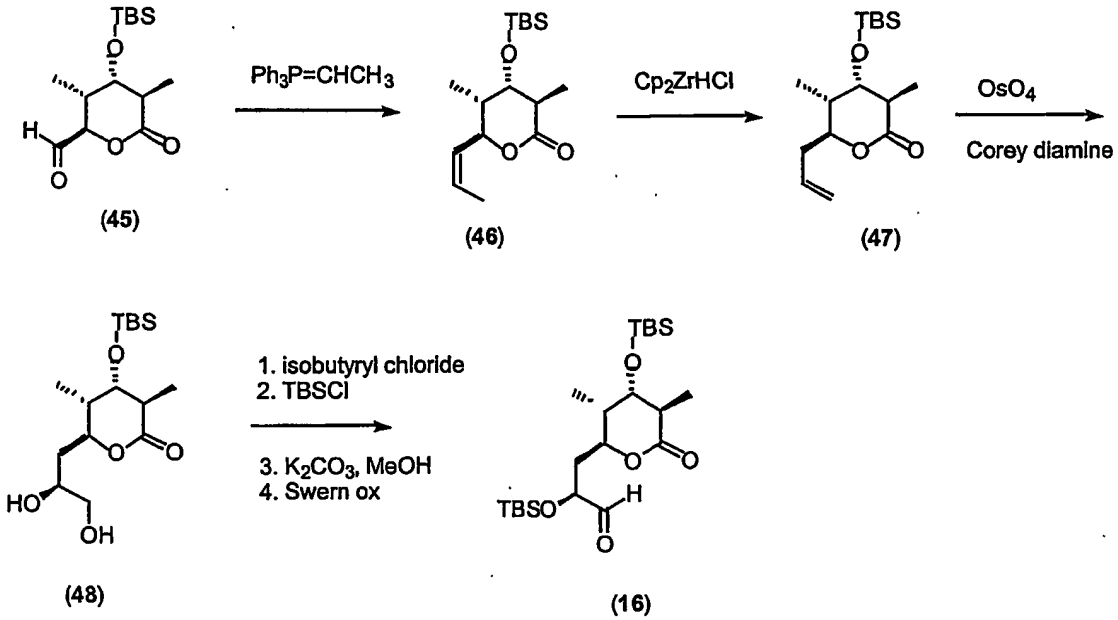
SCHEME 11



(31) (44) (45)

In one embodiment of the invention (Scheme 12), (**45**) is reacted with (ethylidene)triphenylphosphorane, producing compound (**46**). This is treated with bis(cyclopentadienyl)zirconium chloride hydride (Cp_2ZrHCl) to isomerize the alkene to the terminal position. Asymmetric dihydroxylation of the alkene using the stoichiometric osymlation conditions of Corey using a chiral C_2-symmetric diamine ligand [*N,N'*-bis(mesitylmethyl)-(*R,R'*)-1,2-diphenyl-1,2-diaminoethane] yields diol (**47**), which is again differentially protected to allow for oxidation of the terminal alcohol to the aldehyde (**48**). Compound (**48**) is converted into discodermolide according to Smith *et al*, "Gram-scale synthesis of (+)-discodermolide," *Organic Letters* (1999) *1*: 1823-1826.

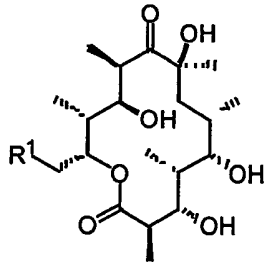
SCHEME 12



(45) (46) (47) (48) (16)

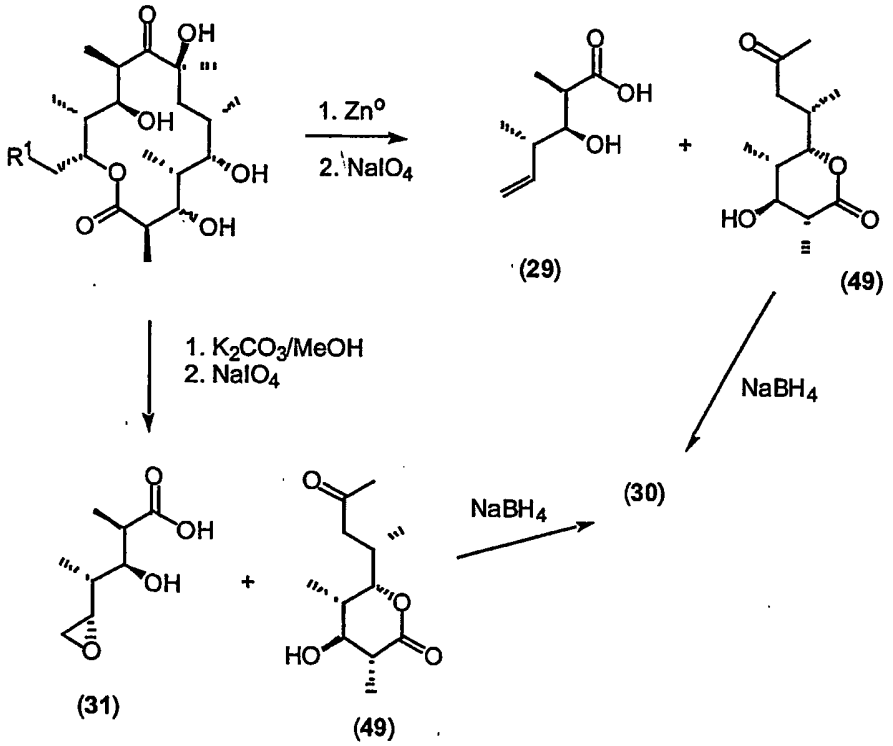
Completion of the synthesis of discodermolide according to the invention follows methods known in the art.

In another embodiment, compounds of formula (F)



wherein R^1 is H, halogen or phenylthio and R^2 = OH, are used to prepare intermediates (29), (30), and (31) described above, as illustrated in Scheme 13.

SCHEME 13



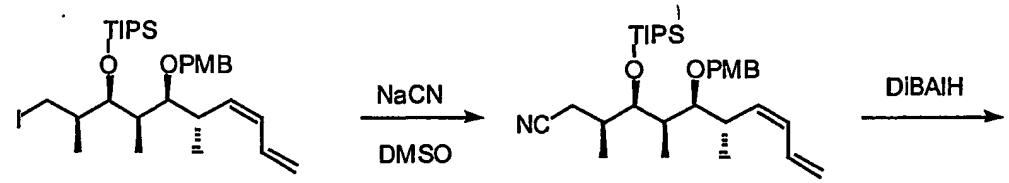
Treatment of (F) wherein R^2 = OH with a reagent capable of cleaving alpha-hydroxyketones, for example sodium periodate, results in fragmentation of the C8-C9 bond. When R^1 = halogen or phenylthio, treatment with zinc as described above causes elimination of the halo lactone to give (29) and (49). When R^1 = H, transesterification using basic methanol is used to cleave the lactone to give (31) and (49). Compound (49) is the ketone analog of (30), and so is converted into (30) by reaction with a reducing agent, such as sodium borohydride.

Discodermolide Compounds with C-14 Modifications

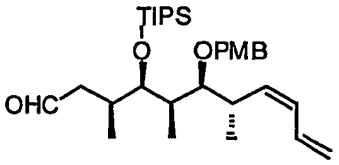
In another aspect of the invention, discodermolides having variations at C-14 are provided. As illustrated in Scheme 10 above, treatment of compound (42) with a Wittig reagent other than (1-iodoethylidene)-triphenylphosphorane yields discodermolides having groups other than methyl at C14. A preferred example of an alternate Wittig reagents is (iodomethylidene)-triphenylphosphorane, which results in the formation of 14-nordiscodermolide when used according to the Smith protocol.

In another embodiment, 14-nordiscodermolide compounds are made by coupling the appropriate fragments through a Wittig olefination rather than the palladium-mediated coupling of Smith *et al.* As shown in Scheme 14, the appropriate "A fragment" is prepared from intermediate (38) by homologation to form aldehyde (51).

SCHEME 14



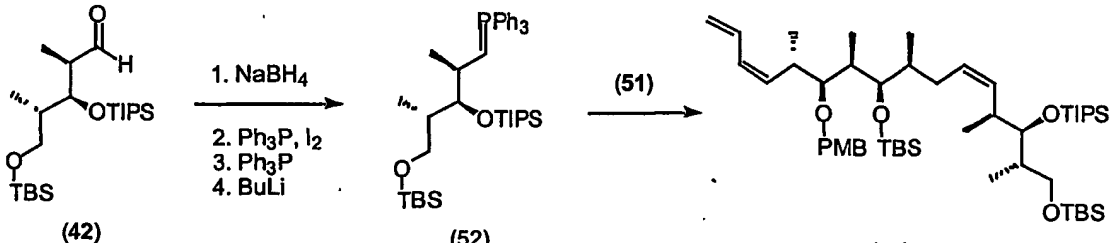
(38) (50)



(51)

Aldehyde (51) is coupled with phosphorus ylid (52), derived from previously-described (42) as shown in Scheme 15 to yield the 14-nordiscodermolide intermediate (53).

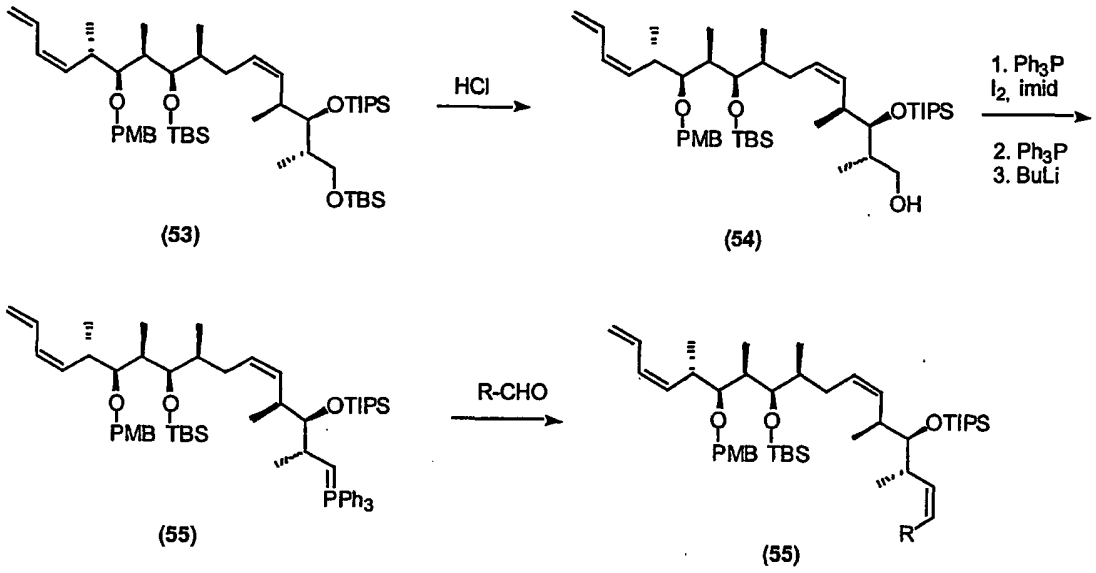
SCHEME 15



(42) (52) (53)

Subsequent conversion of (53) into 14-nordiscodermolide is illustrated in Scheme 16. Selective deprotection using mild acid treatment, for example brief exposure to HCl, yields alcohol (54) which is converted into the phosphonium salt (55) via the iodide. The ylid from (55) is reacted with an aldehyde (R-CHO) comprising the "C fragment" or an analog in order to complete assembly of the discodermolide carbon skeleton.

SCHEME 16



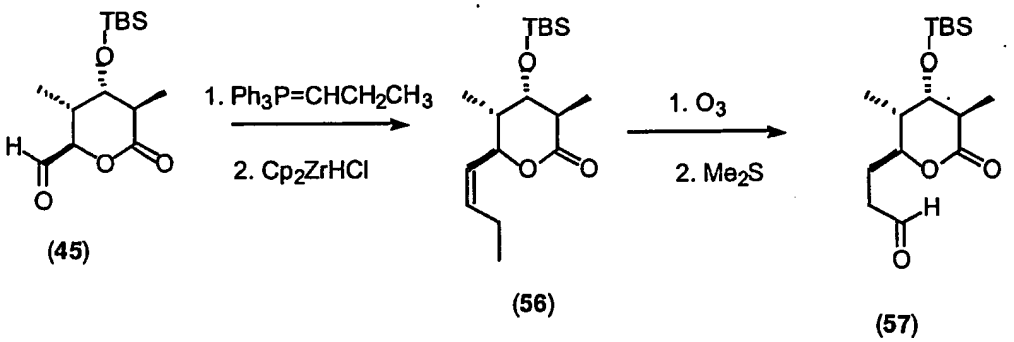
Discodermolide Compounds with "C-fragment" Modifications

The "C-fragment" of discodermolide, comprising C1-C7, is a particularly attractive target for analog production based on known structure-activity relationships. Hydroxyl groups at C-3 and C-7 positions may be converted into other groups such as acyl, alkoxy, aryloxy or other hydroxy protecting groups. Alternatively, the entire "C-fragment" may be replaced by other groups, for example lactams rather than lactones, or by simpler chemical analogs in which one or more of the functionalities present on the "C-fragment" are absent.

In one aspect of the invention, discodermolide compounds having hydrogen at C-3 and/or C-7 position are provided.

In another embodiment, (45) is reacted with (propylidene)triphenylphosphorane as shown in Scheme 17. The resulting alkene is treated with bis(cyclopentadienyl)zirconium hydride chloride to isomerize the alkene to the terminal position. Ozonolysis yields fragment (57), which is used to prepare 7-deoxydiscodermolide.

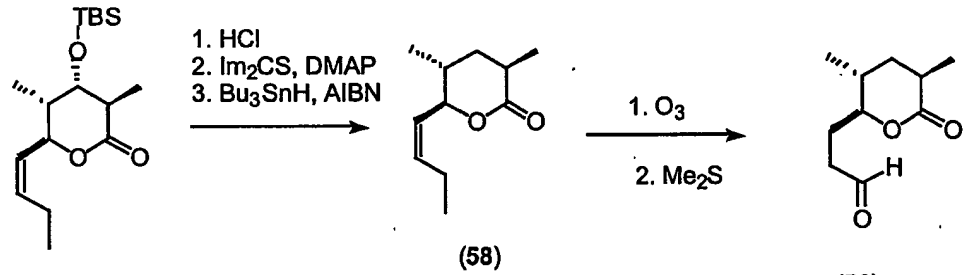
SCHEME 17



(45) (56) (57)

In a second embodiment, compound (56) is deprotected (Scheme 18) and the alcohol is removed by a Barton deoxygenation (thiocarbonyldiimidazole, followed by Bu_3SnH) to yield (58). Subsequent treatment as above yields fragment (59), which is useful in the synthesis of 3,7-dideoxydiscodermolides.

SCHEME 18



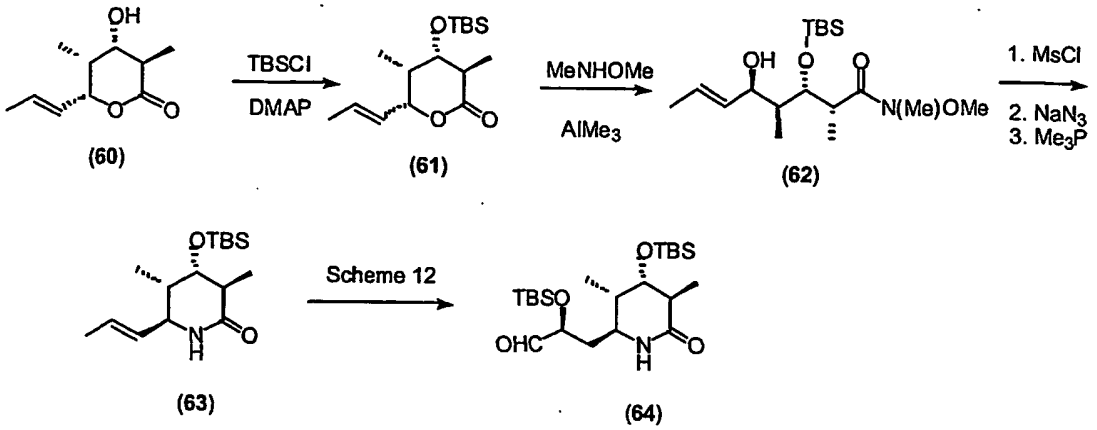
(56) (58) (59)

Lactam Discodermolide Compounds

In another aspect of the invention, fermentation-derived polyketides prepared using genetically-engineered PKSs are used to produce lactam analogs of discodermolides, i.e., 5-deoxy-5-aminodiscodermolides.

In one embodiment, lactam discodermolides are made using compound (64). One method for making (64) is outlined by Scheme 19.

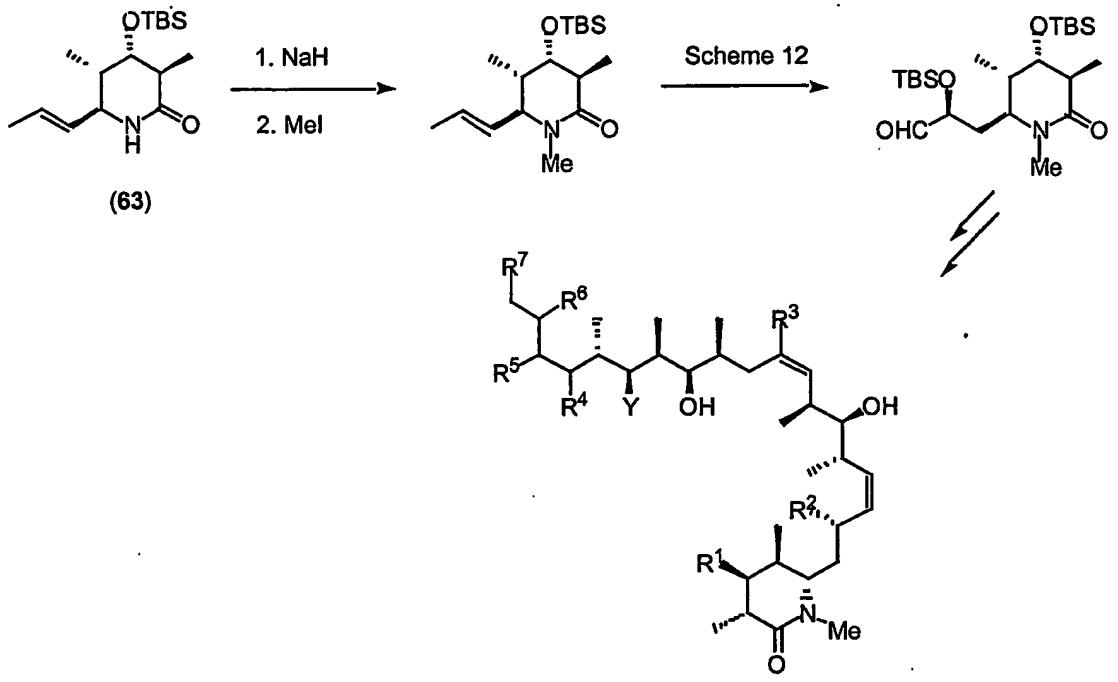
SCHEME 19



Lactone (E) wherein R^1 = methyl and R^2 = H (**60**) is prepared as described above. Protection of the alcohol, preferably as the TBS silyl ether, followed by delactonization yields Weinreb amide (**62**). The allylic alcohol of (**62**) is activated, preferably as the mesylate, and displaced with inversion of configuration using azide. Staudinger reduction using trimethylphosphine gives lactam (**63**), which is converted into lactam fragment (**64**) according to the methods shown in Scheme 12 above.

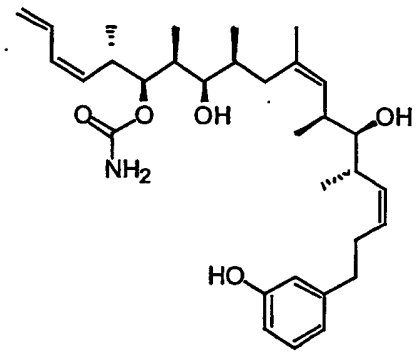
In another embodiment of the invention, the nitrogen of lactam (**63**) is optionally alkylated by treatment with a stron base, preferably NaH, followed by reaction with a alkylating agent, for example methyl iodide (Scheme 20). In this manner, N-alkyl discodermolides are prepared.

SCHEME 20



Any of the above-described compounds (**57**), (**59**), (**64**), and the like can replace the Smith "C-fragment" (**16**) in any of the previously described reactions to make discodermolide compounds, including 14-nordiscodermolides. Further, use of even simpler aldehydes in place of the "C-fragment" will give rise to further discodermolide analogs. Examples of such discodermolide analogs comprising simplified C-fragments are given in Hirokazu *et al.*, "Compounds which mimic the chemical and biological properties of discodermolide," PCT publication WO01/42179 (incorporated herein by reference).

A particularly preferred embodiment of the invention includes discodermolide analogs in which the "C-fragment" is replaced by a (3-hydroxyphenyl)ethyl group, such as compound (**104**).

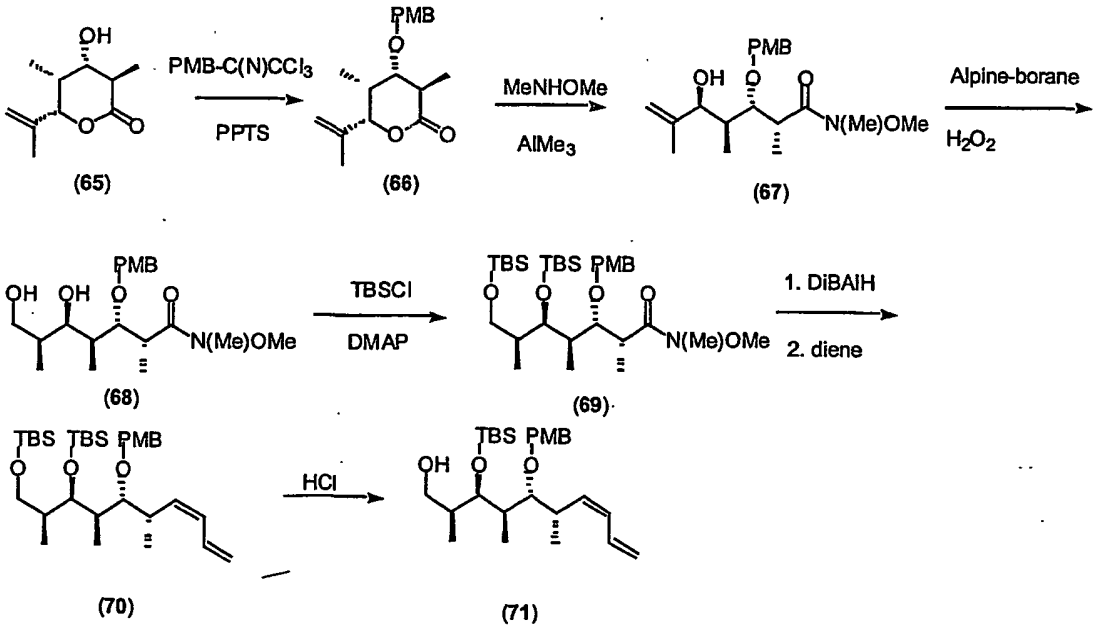


(104)

Compound (104) is prepared as described in Scheme 16 above, wherein "R-CHO" is 3-(3-*tert*-butyldimethylsilyloxy)phenylpropanal.

Discodermolide Compounds where Y is amino or -NHC(=O)NH_2.

In another aspect of the invention, discodermolide compounds where Y is amino or -NHC(=O)NH_2 are made using compound (71), a modified version of compound (37) in which the carbon center containing the PMB protected alcohol is the opposite stereochemistry. Compound (71) is prepared from fermentation-derived lactone (65), a compound of formula (E) wherein R^1 = H and R^2 = methyl prepared using the (ery)[module(1)-KS°]-[module(2)]-TE PKS (Scheme 21).SCHEME 21

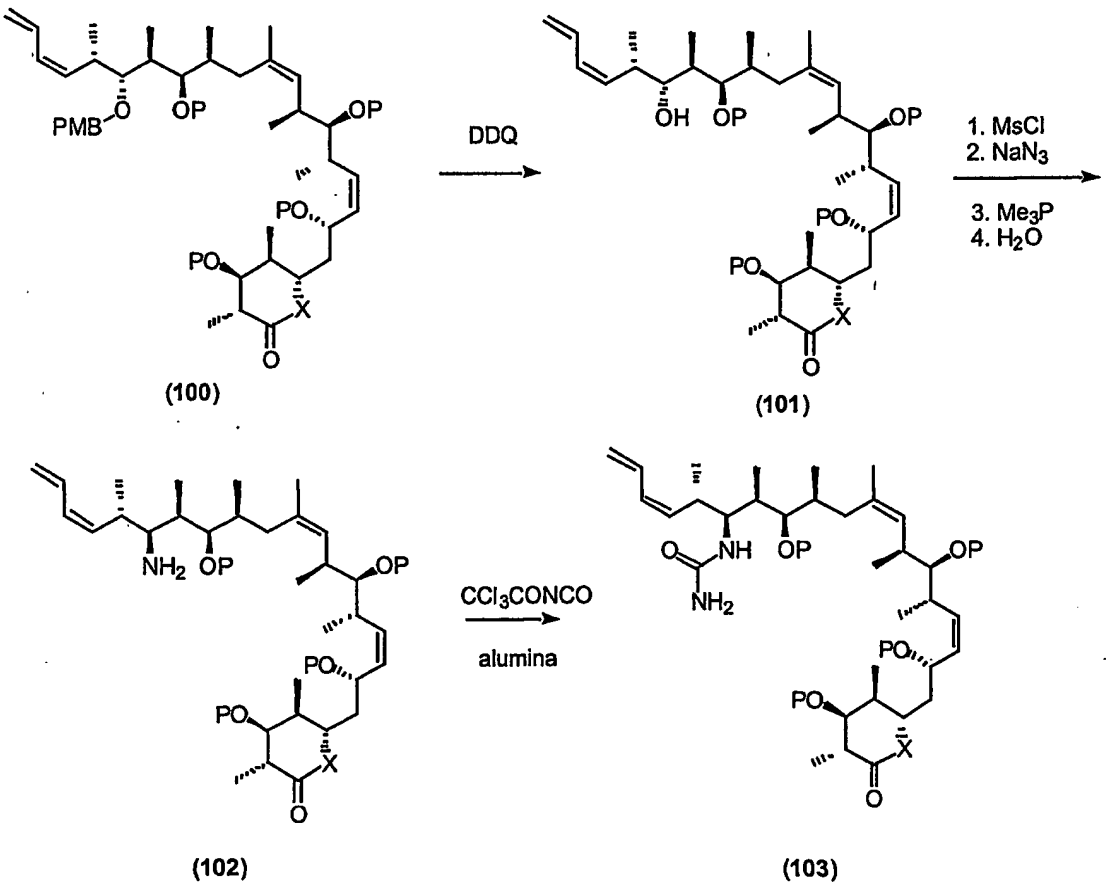


Lactone (65) is protected on the hydroxyl as the PMB ether, using PMB trichloroacetimidate and an acid catalyst, then is converted into Weinreb amide (67).

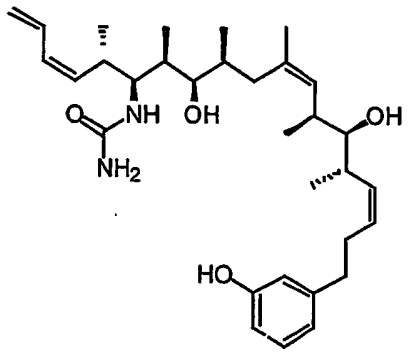
Stereoselective hydroboration using Alpine-Borane followed by an oxidative workup gives diol (68), which is protected as the bis-TBS ether (69). Reduction of the Weinreb amide to the aldehyde allows installation of the diene segment of discodermolide as described in Scheme 7 above. Selective deprotection of the primary alcohol provides (71).

Compound (71) may be combined with other compounds of the invention to prepare discodermolide derivatives having a hydroxyl at Y where the carbon containing Y (C19) is of the opposite stereochemistry that is normally found in discodermolide (100), including 14-nordiscodermolides and discodermolides having further modifications to the C-segment, such as deoxy analogs and lactams These compounds are converted into the 19-aminodiscodermolides by removal of the PMB ether (dichlorodicyanoquinone, DDQ) to give the alcohol (101), activation of the hydroxyl as the mesylate, displacement of the mesylate by azide with inversion of configuration at C19, and finally Staudinger reduction of the azide to the amine to give (102) (Scheme 22).

SCHEME 22



The amino group of compound **(102)** may be further modified using any number of standard reactions known in the art. In one preferred embodiment, the amino group of compound **(102)** is converted into an urea group to yield 19-urea compound **(103)** as shown in Scheme 21. In another preferred embodiment, the 19-urea analog **(105)** of compound **(104)** is prepared according to the methods of the invention.



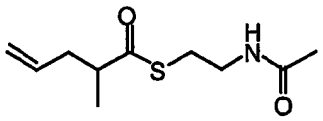
(105)

As illustrated by the above epothilone and discodermolide examples, there are a variety of methods to make a particular polyketide using chimeric PKS constructs of the invention. A number of alternate synthetic schemes are presented in U.S. patent Application No. 60/224,038 filed August 9, 2000 by inventors Daniel Santi and Gary Ashley entitled BIO-INTERMEDIATES FOR USE IN THE CHEMICAL SYNTHESIS OF POLYKETIDES (incorporated herein by reference). Numerous examples of these alternate procedures are given in the following Examples. Although the library of polyketide structures may be amended as additional polyketides become known, a sufficient diversity exists in the current library to make almost any polyketide structure using purely biological techniques. The number and diversity of the polyketides that may be made increases dramatically when the biological techniques described herein are combined with standard chemical strategies.

A detailed description of the invention having been provided above, the following examples are given for the purpose of illustrating the present invention and shall not be construed as being a limitation on the scope of the invention or claims.

EXAMPLE 1

2-methyl-4-pentenoate N-acetylcysteamine thioester

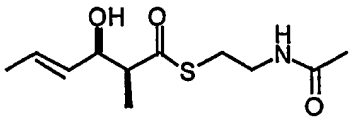


A solution of 2-methyl-4-pentenoic acid (1.14 g) and diphenyl phosphorylazide (3.0 g) in 10 mL of dry tetrahydrofuran cooled on ice was treated with 5 mL of dry triethylamine and allowed to stir for 30 minutes under inert atmosphere. Freshly distilled N-acetylcysteamine (1.5 g) was added and the mixture was allowed to stir for an additional hour. The reaction was quenched by addition of 10 mL of 2N HCl, diluted with 50 mL of ethyl acetate, and the phases were separated. The organic phase was washed sequentially with water and brine, then dried over $MgSO_4$, filtered, and evaporated to yield the crude thioester as a yellow oil. Purification by silica gel chromatography (ether) afforded pure product as an oil.

^{1}H-NMR ($CDCl_3$, 400 MHz): δ 5.96 (1H, br s), 5.72 (1H, ddt, J = 7.2,10.0,17.2 Hz), 5.07 (1H,dm, J = 17.2 Hz), 5.04 (1H,dm,J = 10.0 Hz), 3.42 (2H, m), 3.02 (2H,m), 2.74 (1H,sextet,J=7), 2.44 (1H,m), 2.18 (1H,m), 1.96 (3H,s), 1.90 (3H,d,J = 7 Hz). ^{13}C-NMR ($CDCl_3$, 100 MHz): δ 203.68, 170.49, 134.85, 117.25, 48.17, 39.76, 38.03, 28.20, 23.17, 17.13.

EXAMPLE 2

(±)-*syn*-2-methyl-3-hydroxy-4-hexenoate N-acetylcysteamine thioester



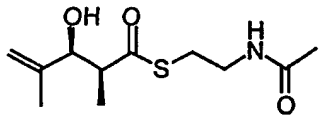
Step 1. A solution of N-propionyl-2-benzoxazolone (100.0 g) in anhydrous CH_2Cl_2 (1100 mL) was cooled to 3 °C with mechanical stirring under N_2 atmosphere. $TiCl_4$ (58.4 mL) was added at a rate such that the internal temperature remained below 10 °C (ca. 10 minutes). The resulting yellow slurry was stirred vigorously for 40 minutes, then triethylamine (87.4 mL) was added at a rate such that the internal temperature remained

below 10 °C (ca. 10 minutes). The resulting deep red solution was stirred for 80 minutes. Crotonaldehyde (55 mL) was added at a rate such that the internal temperature remained below 10 °C (ca. 20 minutes), and the reaction was followed by thin-layer chromatography (4:1 hexanes/ethyl acetate). After stirring for 90 minutes, the reaction was quenched by addition of 450 mL of 2 N HCl. The phases were separated, and the aqueous phase was extracted 3 times with 750-mL portions of ether. The organic phases were combined and washed three times with 200-mL portions of 2 N HCl. The acidic washes were combined and back-extracted 3 times with 150-mL portions of ether. The combined organic extract was washed once with 300 mL of sat. aq. $NaHCO_3$, and once with 300 mL of sat. aq. NaCl. The organic phase was then dried over $MgSO_4$, filtered, and concentrated under vacuum to a yellow slurry. The product was collected by vacuum filtration and rinsed with hexanes to yield a colorless solid. Concentration of the filtrate yielded a second crop of product, which was collected in the same manner, giving a combined 103 g (80% yield) of crystalline product; mp = 123-4 °C. The mother liquor can be chromatographed (4:1 hexanes/ethyl acetate) to yield additional product. mp 74-6 °C. ^{1}H NMR ($CDCl_3$) δ 8.06 (m, 1 H); 7.23 (m, 3 H); 5.78 (dqd, 1 H, J = 15, 7, 1 Hz); 5.55 (ddq, 1 H, J = 15, 7, 2 Hz); 4.52 (br, 1 H); 4.05 (qd, 1 H, J = 7, 4 Hz); 2.38 (br d, 1 H, J = 3 Hz); 1.70 (ddd, 3 H, J = 7, 1, 1 Hz, 3 H); 1.30 (d, 3 H, J = 7 Hz). ^{13}C NMR ($CDCl_3$) δ 175.50, 151.20, 142.18, 129.99, 128.78, 127.80, 125.43, 124.86, 116.22, 109.85, 72.92, 44.31, 17.71, 11.04.

Step 2. One molar equivalent of sodium methoxide (25% w/v in methanol; ca. 150 mL) is added in a slow stream to a solution of N,S-diacetylcysteamine (173 g) in methanol (910 mL) under N_2. When half of the calculated volume has been added, the reaction is monitored by TLC (1:1 ethyl acetate/hexanes), and methoxide addition is continued until complete conversion of the N,S-diacetylcysteamine to N-acetylcysteamine. Acetic acid (50 g) is added, and the resulting solution of sodium thiolate is cannulated into a flask containing solid (±)-N-[*syn*-2-methyl-3-hydroxy-4-hexenoyl]-2-benzoxazolone (240 g) under N_2. After 15 minutes, the reaction is quenched with solid oxalic acid dihydrate (80.4 g), filtered, and concentrated to a yellow oil. The residue is dissolved in 2:1 hexanes/ethyl acetate and submitted to batch elution chromatography on SiO_2. The silica is washed with 2:1 hexanes/ethyl acetate to remove 2-benzoxazolone, then with ethyl acetate/methanol (9:1) to elute the product thioester. Evaporation of the thioester-containing eluent yields the product.

EXAMPLE 3

(±)-*syn*-4-methyl-3-hydroxy-4-hexenoate N-acetylcysteamine thioester



Step 1. A solution of N-propionyl-2-benzoxazolone (100.0 g) in anhydrous CH_2Cl_2 (1100 mL) is cooled to 3 °C with mechanical stirring under N_2 atmosphere. $TiCl_4$ (58.4 mL) is added at a rate such that the internal temperature remains below 10 °C (ca. 10 minutes). The resulting yellow slurry is stirred vigorously for 40 minutes, then triethylamine (87.4 mL) is added at a rate such that the internal temperature remains below 10 °C (ca. 10 minutes). The resulting deep red solution is stirred for 80 minutes. Methacrolein (55 mL) is added at a rate such that the internal temperature remains below 10 °C (ca. 20 minutes), and the reaction is followed by thin-layer chromatography (4:1 hexanes/ethyl acetate). After stirring for 90 minutes, the reaction is quenched by addition of 450 mL of 2 N HCl. The phases are separated, and the organic phase is filtered through a pad of silica gel. The silica gel is washed with ether, and the combined organic are concentrated under vacuum to a. The product is collected by vacuum filtration and rinsed with hexanes to yield a colorless solid.

Step 2. One molar equivalent of sodium methoxide (25% w/v in methanol; ca. 150 mL) is added in a slow stream to a solution of N,S-diacetylcysteamine (173 g) in methanol (910 mL) under N_2. When half of the calculated volume has been added, the reaction is monitored by TLC (1:1 ethyl acetate/hexanes), and methoxide addition is continued until complete conversion of the N,S-diacetylcysteamine to N-acetylcysteamine. Acetic acid (50 g) is added, and the resulting solution of sodium thiolate is cannulated into a flask containing solid (±)-N-[*syn*-4-methyl-3-hydroxy-4-hexenoyl]-2-benzoxazolone (240 g) under N_2. After 15 minutes, the reaction is quenched with solid oxalic acid dihydrate (80.4 g), filtered, and concentrated to a yellow oil. The residue is dissolved in 2:1 hexanes/ethyl acetate and submitted to batch elution chromatography on SiO_2. The silica is washed with 2:1 hexanes/ethyl acetate to remove 2-benzoxazolone, then with ethyl acetate/methanol (9:1) to elute the product thioester. Evaporation of the thioester-containing eluent yields the product.

EXAMPLE 4

General Procedure for Polyketide Production by Fermentation

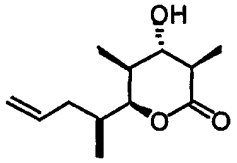
Cultures are grown at 30°C and 150-250 rpm in FKA basal medium (45 g/L starch, 10 g/L corn steep liquor, 10 g/L dried debittered brewer's yeast, 1 g/L calcium carbonate, and 23.8 g/L HEPES, free acid) (Sigma-Aldrich, St. Louis, MO). Shake flask medium pH is adjusted to pH 7.0 prior to sterilization by autoclaving for 90 min at 121°C. Bioreactor fermentation medium is prepared without HEPES buffer and autoclaved for 90 min at 121°C. After sterilization and cooling, the medium was adjusted to pH 6.5. All media are supplemented with 50 mg/L thiostrepton (Calbiochem, La Jolla, CA) in (50 mg/mL) DMSO and 10 mL/L of 50% (v/v) antifoam (Antifoam B, J.T.Baker, Phillipsburg, NJ) as post-sterile additions. Strains are maintained as frozen cell banks prepared by adding glycerol (30% v/v final) to an exponentially growing culture (in FKA medium) and freezing 1 mL aliquots at –85°C. Thioester feedstocks (400 mg/mL) and thiostrepton (50 mg/mL) are prepared as DMSO solutions which were sterile filtered using 0.2μm nylon membranes before addition to cultures.

Primary seed cultures are established by inoculating 50 mL of FKA with a cell bank vial and cultivating for 3 days. Glucose feeding is performed by the addition of a sterile filtered 50% (w/v) glucose solution as a single daily bolus to give the desired feed rate. Bioreactor fermentations are performed in B. Braun MD 5L fermentors with 3 L of production medium operated at 30°C, 0.3 VVM airflow, and 600 rpm agitation. Dissolved oxygen concentration and pH are monitored using autoclaveable electrodes (Mettler Toledo, Wilmington, MA). Under these operating conditions, dissolved oxygen is maintained above 50% at all times. Foaming is controlled by automatic addition of 50% (v/v) Antifoam B solution. The pH is controlled by automatic addition of 2.5 N sodium hydroxide or sulfuric acid. Bioreactors are inoculated with 5% (v/v) secondary seed culture prepared by sub-culturing 25 mL of primary seed into 500 mL of FKA and cultivation for 2 days. Upon inoculation into the bioreactors, the thioester feedstock is added to a final concentration of 2 g/L, and the fermentation is allowed to proceed for 6 days. The cells are removed by centrifugation, and the broth is filtered through a column of XAD-16 to absorb polyketide products. After washing with 2 column volumes of water, the resin is eluted

with acetone. The eluate is evaporated to an aqueous slurry, which is extracted with ethyl acetate. The extract is dried and concentrated. The polyketide product is purified by silica gel chromatography.

EXAMPLE 5

(2*R*,3*S*,4*S*,5*S*,6*S*)-3,5-dihydroxy-2,4,6-trimethyl-8-nonenoate δ-lactone



Method A:

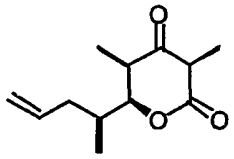
Streptomyces lividans K4-155 was transformed with plasmid pKOS10-153 containing the gene encoding the DEBS3 protein in a pSET type (an integrative) plasmid. An agar plug was used to inoculate a 5 mL seed culture of R6 broth and was grown for 2 days at 30 °C at 200 rpm. A 2.5 mL portion of this culture was used to inoculate 50 mL of R6 media in a 250 mL baffled flask and was incubated at 30 ° at 200 rpm. After 1 day, a solution of 2-methyl-4-pentenoate N-acetylcysteamine thioester was added (to a final concentration of 1 g/L from a 40% w/v solution in DMSO). Samples of broth were then taken from the flasks at intervals. The broth from cultures with and without the addition of thioester were analyzed by LC-MS.

Method B:

A solution of 2-methyl-4-pentenoate N-acetylcysteamine thioester (20 g/L) in methylsulfoxide is added to a 2-day old culture of *Streptomyces coelicolor* CH999 harboring a plasmid which contains the gene encoding the DEBS3 protein. The fermentation is performed as described until the general procedure of Example 4.

EXAMPLE 6

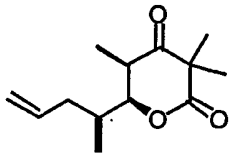
(*2R,4R,5S,6S*)-5-hydroxy-3-oxo-2,4,6-trimethyl-8-nonenoate δ-lactone



Dry methylsulfoxide (0.45 mL) is added dropwise to a solution of oxalyl chloride (0.28 mL) in 30 mL of CH_2Cl_2 under inert atmosphere at –78 °C. After 10 minutes, a solution of (*2R,3S,4S,5S,6S*)-3,5-dihydroxy-2,4,6-trimethyl-8-nonenoate δ-lactone (600 mg) in 10 mL of CH_2Cl_2 is added dropwise, followed by triethylamine (2.0 mL). Stirring is continued for 20 minutes, then the reaction is allowed to warm to ambient temperature over 30 minutes. Brine (30 mL) is added and the mixture is extracted with CH_2Cl_2. The organic extracts are combined, washed with brine, dried over $MgSO_4$, filtered, and evaporated. The product is purified by silica chromatography.

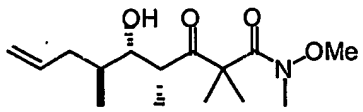
EXAMPLE 7

(*4R,5S,6S*)-5-hydroxy-3-oxo-2,2,4,6-tetramethyl-8-nonenoate δ-lactone



A solution of (*2R,4R,5S,6S*)-5-hydroxy-3-oxo-2,4,6-trimethyl-8-nonenoate δ-lactone (2.1 g) in 10 mL of tetrahydrofuran is added dropwise to a 0 °C suspension of sodium hydride (0.50 g of a 60% dispersion in oil) in 10 mL of tetrahydrofuran. Methyl iodide (2 g) is added, and the mixture is warmed to ambient temperature and stirred until complete as evidenced by thin layer chromatographic analysis. The reaction is diluted with ether, quenched by addition of 1 N HCl at 0 °C, and the phases are separated. The organic phase is washed with brine, dried over $MgSO_4$, filtered, and evaporated. The crude product is purified by silica gel chromatography.

EXAMPLE 8

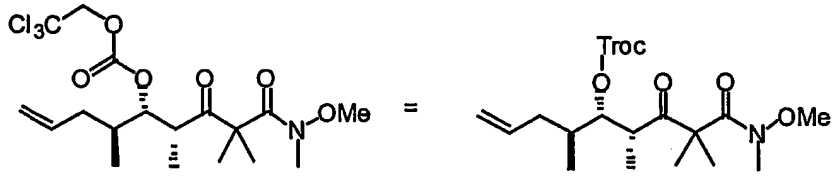


N-Methyl-N-methoxy (*4R,5S,6S*)-5-hydroxy-3-oxo-2,4,6-tetramethyl-8-nonenoate amide

A solution of 2 M trimethylaluminum in toluene (10 mmol) is added dropwise to a suspension of N,O-dimethylhydroxylamine hydrochloride (10 mmol) in 8 mL of CH_2Cl_2 at 0 °C. The resulting homogeneous solution is stirred for 30 min at ambient temperature. A solution of (*4R,5S,6S*)-5-hydroxy-3-oxo-2,2,4,6-tetramethyl-8-nonenoate □-lactone (2 mmol) in 4 mL of CH_2Cl_2 is added over 10 min, and the resulting solution is heated at reflux until complete consumption of starting material. Upon cooling, the mixture is poured into 1 M HCl, and extracted with CH_2Cl_2. The extract is washed sequentially with 5% $NaHCO_3$ and brine, dried over $MgSO_4$, filtered, and evaporated. The product is purified by silica gel chromatography.

EXAMPLE 9

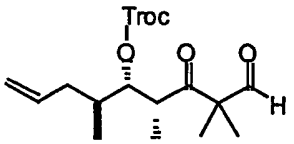
N-Methyl-N-methoxy (*4R,5S,6S*)-5-((2,2,2-trichloroethoxy)carbonyloxy)-3-oxo-2,4,6-tetramethyl-8-nonenoate amide



A solution of N-Methyl-N-methoxy (*4R,5S,6S*)-5-hydroxy-3-oxo-2,4,6-tetramethyl-8-nonenoate amide (1 mmol) and 2,2,2-trichloroethyl chloroformate (1.5 mmol) in pyridine (2 mL) is stirred overnight. The mixture is concetrated to dryness. The residue is redissolved in CH_2Cl_2 and washed sequentially with 1 N HCl, 5% $NaHCO_3$, and brine. After drying over $MgSO_4$, the solution is filtered and evaporated to dryness. The product is purified by silica gel chromatography.

EXAMPLE 10

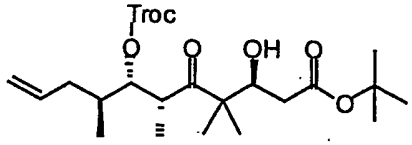
(*4R,5S,6S*)-5-((2,2,2-trichloroethoxy)carbonyloxy)-3-oxo-2,4,6-tetramethyl-8-nonenal



A solution of N-Methyl-N-methoxy (*4R,5S,6S*)-5-((2,2,2-trichloroethoxy)carbonyloxy)-3-oxo-2,4,6-tetramethyl-8-nonenoate amide (10 mmol) in 20 mL of toluene is cooled to –78 °C, and a 1.5 M solution of diisobutylaluminum hydride in toluene (50 mmol) is added dropwise over a 45 minute period. Stirring is continued until complete conversion of starting material as determined by thin-layer chromatographic analysis. Ethyl acetate (10 mL) is added, and the mixture is allowed to warm to ambient temperature. A 100-mL portion of 1 N HCl is added, and the mixture is stirred an additional 1 hour before extracting with CH_2Cl_2. The extract is dried over $MgSO_4$, filtered, and evaporated. The product is purified by silica gel chromatography.

EXAMPLE 11

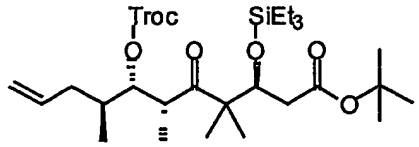
Tert-butyl (3S,6R,7S,8S)-3-hydroxy-5-oxo-4,4,6,8-tetramethyl-7-(2,2,2-trichloroethoxycarbonyl)-10-undecenoate



Tert-butyl acetate (1.0 mL) is added to a –78 °C solution of lithium diisopropylamide (8.7 mmol) in 35 mL of ether, and the mixture is tirred for 1 hour. A solution of bis[1,2:5,6-di-*O*-isopropylidene-α-L-glucofuranos-3-*O*-yl]cyclopentadienyltitanium chloride (9.7 mmol) in 100 mL of ether is added over 1 hour, and stirring is continued for an additional 30 minutes. The reaction is allowed to warm to –30 °C, kept 1 hour, then cooled to –78 °C. A solution of (*4R,5S,6S*)-5-((2,2,2-trichloroethoxy)carbonyloxy)-3-oxo-2,4,6-tetramethyl-8-nonenal in 20 mL of ether is added and the reaction is stirred for 2 hours, quenched with aqueous THF, then filtered through Celite and concentrated. The product is isolated by silica gel chromatography using 7% ethyl acetate in hexanes.

EXAMPLE 12

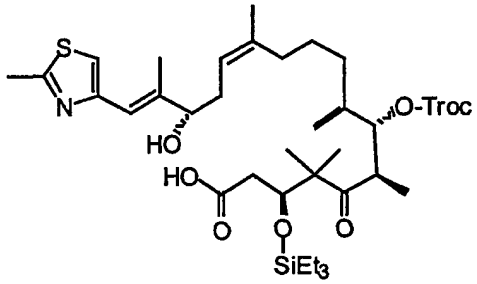
Tert-butyl (3S,6R,7S,8S)-3-(triethylsilyloxy)-5-oxo-4,4,6,8-tetramethyl-7-(2,2,2-trichloroethoxycarbonyl)-10-undecenoate



A mixture of *Tert*-butyl (3S,6R,7S,8S)-3-hydroxy-5-oxo-4,4,6,8-tetramethyl-7-(2,2,2-trichloroethoxycarbonyl)-10-undecenoate (1.0 g), triethylchlorosilane (0.38 g), and imidazole (0.27 g) in 5 mL of dimethylformamide is stirred overnight, then poured into water and extracted with ether. The extract is evaporated and chromatographed on silica gel to yield the product.

EXAMPLE 13

(*3R,6R,7S,8S*)- 15-hydroxy-3-(triethylsilyloxy)-5-oxo-4,4,6,8,12,16-hexamethyl-7-((2,2,2-trichloroethoxy)carbonyloxy)-17-(2-methyl-4-thiazolyl)heptadeca-12,16-dienoic acid

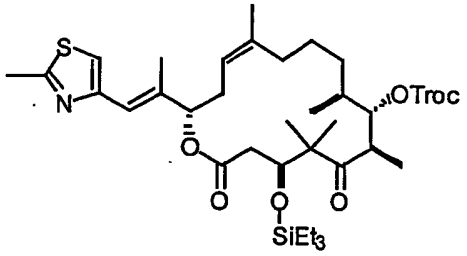


A solution of ^{t}butyl (*3R,6R,7S,8S*)- 3-(triethylsilyloxy)-5-oxo-4,4,6,8-tetramethyl-7-((2,2,2-trichloroethoxy)carbonyloxy)-10-undecenoate thioester (10 mmol) in 80 mL of tetrahydrofuran is treated with a 0.5 M solution of 9-borabicyclo[3.3.1]nonane in tetrahydrofuran (30 mL). In a separate flask, 4-(6-iodo-3-acetoxy-2-methyl-2,6,-heptadienyl)-2-methylthiazole (13 mmol) is dissolved in 100 mL of dimethylformamide, and cesium carbonate (21 mmol) is added with vigorous stirring followed by sequential addition of triphenylarsine (11 mmol), [1,1'-bis(diphenylphosphino)ferrocene]-dichloropalladium(II) (1 mmol), and water (7 mL). After 4 hours, the borane mixture is added to the iodide mixture. After the color fades (ca. 2 hours), the mixture is poured into water, the pH is adjusted to 4.0 using 1 N HCl, and the mix is extracted with ether. The extract is washed sequentially with water and brine, dried over $MgSO_4$, filtered, and evaporated. The crude material is dissolved in 1:1 methanol/water and stirred with

potassium carbonate to remove the acetate and thioesters. Upon completion, the mixture is concentrated to remove methanol, and the aqueous phase is adjusted to pH 4 with 1 N HCl prior to extraction with ether. The extract is dried over $MgSO_4$, filtered, and evaporated. The product is isolated by silica gel chromatography.

EXAMPLE 14

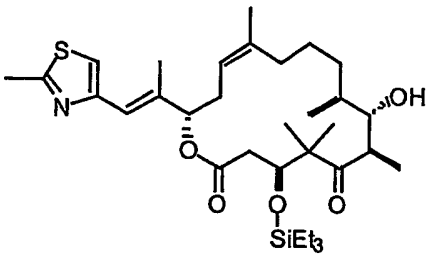
3-O-triethylsilyl-7-O-(2,2,2-trichloroethoxycarbonyl)epothilone D



A solution of (*3R,6R,7S,8S*)- 15-hydroxy-3-(triethylsilyloxy)-5-oxo-4,4,6,8,12,16-hexamethyl-7-((2,2,2-trichloroethoxy)carbonyloxy)-17-(2-methyl-4-thiazolyl)heptadeca-12,16-dienoic acid (10 mmol) in tetrahydrofuran (150 mL) is treated with triethylamine (60 mmol) and 2,4,6-trichlorobenzoylchloride (50 mmol) for 15 minutes at ambient temperature, then diluted with 800 mL of toluene. This solution is added dropwise to a stirred solution of 4-dimethylaminopyridine (105 mmol) in 12 L of toluene. After addition, the mix is stirred for an additional 1 hour, then concentrated under vacuum. The product is purified by silica gel chromatography.

EXAMPLE 15

3-O-(triethylsilyl)epothilone D

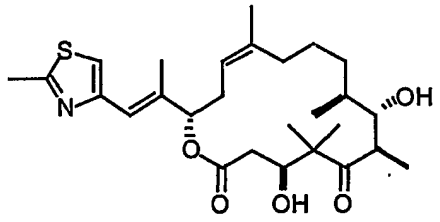


Samarium iodide is prepared by stirring a solution of samarium (3.43 mmol) and iodine (3.09 mmol) in 40 mL of tetrahydrofuran at reflux for 2.5 hours. Upon cooling to ambient

temperature, 10 mg of NiI_2 is added and the mix is cooled to –78 °C. A solution of 3-O-triethylsilyl-7-O-(2,2,2-trichloroethoxycarbonyl)epothilone D (0.386 mmol) in 10 mL of tetrahydrofuran is added, and the mix is stirred for 1 hour at –78 °C. The reaction is quenched by addition of sat. $NaHCO_3$, warmed to ambient temperature, and extracted with ether. The extract is dried over $MgSO_4$, filtered, and evaporated. The product is purified by silica gel chromatography.

EXAMPLE 16

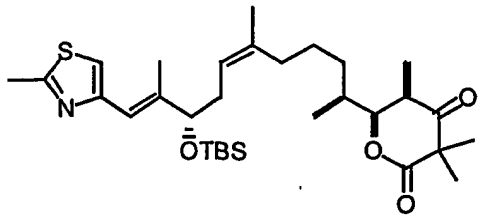
Epothilone D



A solution of 3-O-(triethylsilyl)epothilone D (1 mmol) in 20 mL of tetrahydrofuran in a telfon reaction vessel is cooled on ice and treated with 10 mL of HF•pyridine for 90 min. The reaction is poured into sat. $NaHCO_3$ and extracted with CH_2Cl_2. The extract is dried over $MgSO_4$, filtered, and evaporated. The product is purified by silica gel chromatography.

EXAMPLE 17

(4R,5S,6S,13S)-5-hydroxy-3-oxo-2,2,4,6,10,14-hexamethyl-13-(^{t}butyldimethylsilyloxy)-15-(2-methyl-4-thiazolyl)pentadeca-10,14-dienoic acid δ-lactone

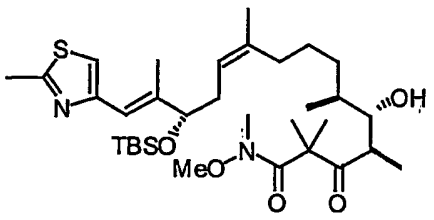


A solution of *(4R,5S,6S)*-5-hydroxy-3-oxo-2,2,4,6-tetramethyl-8-nonenoate δ-lactone (10 mmol) in 80 mL of tetrahydrofuran is treated with a 0.5 M solution of 9-borabicyclo[3.3.1]nonane in tetrahydrofuran (30 mL). In a separate flask, 4-(6-iodo-3-(^{t}butyldimethylsilyloxy)-2-methyl-2,6,-heptadienyl)-2-methylthiazole (13 mmol) is

dissolved in 100 mL of dimethylformamide, and cesium carbonate (21 mmol) is added with vigorous stirring followed by sequential addition of triphenylarsine (11 mmol), [1,1'-bis(diphenylphosphino)ferrocene]-dichloropalladium(II) (1 mmol), and water (7 mL). After 4 hours, the borane mixture is added to the iodide mixture. After the color fades (ca. 2 hours), the mixture is poured into water and extracted with ether. The extract is washed sequentially with water and brine, dried over $MgSO_4$, filtered, and evaporated. The product is isolated by silica gel chromatography.

EXAMPLE 18

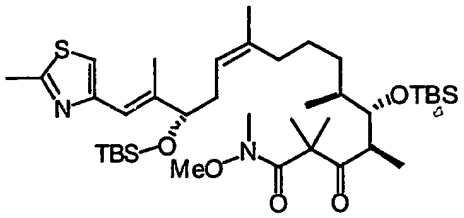
N-methoxy-N-methyl (*4R,5S,6S,13S*)-5-hydroxy-3-oxo-2,2,4,6,10,14-hexamethyl-13-(^{t}butyldimethylsilyloxy)-15-(2-methyl-4-thiazolyl)pentadeca-10,14-dienamide



A solution of 2 M trimethylaluminum in toluene (10 mmol) is added dropwise to a suspension of N,O-dimethylhydroxylamine hydrochloride (10 mmol) in 8 mL of CH_2Cl_2 at 0 °C. The resulting homogeneous solution is stirred for 30 min at ambient temperature. A solution of (*4R,5S,6S,13S*)-5-hydroxy-3-oxo-2,2,4,6,10,14-hexamethyl-13-(^{t}butyldimethylsilyloxy)-15-(2-methyl-4-thiazolyl)pentadeca-10,14-dienoic acid □-lactone (2 mmol) in 4 mL of CH_2Cl_2 is added over 10 min, and the resulting solution is heated at reflux until complete consumption of starting material. Upon cooling, the mixture is poured into 1 M HCl, and extracted with CH_2Cl_2. The extract is washed sequentially with 5% $NaHCO_3$ and brine, dried over $MgSO_4$, filtered, and evaporated. The product is purified by silica gel chromatography.

EXAMPLE 19

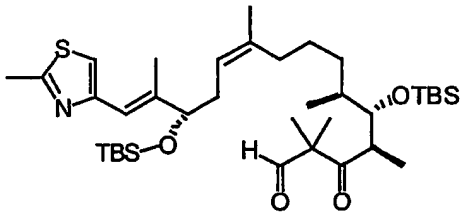
N-methoxy-N-methyl (*4R,5S,6S,13S*)-5-hydroxy-3-oxo-2,2,4,6,10,14-hexamethyl-13,15-di(^{t}butyldimethylsilyloxy)-15-(2-methyl-4-thiazolyl)pentadeca-10,14-dienamide



A solution of N-methoxy-N-methyl (*4R,5S,6S,13S*)-5-hydroxy-3-oxo-2,2,4,6,10,14-hexamethyl-13-(^{t}butyldimethylsilyloxy)-15-(2-methyl-4-thiazolyl)pentadeca-10,14-dienamide (10 mmol) in 50 mL of CH_2Cl_2 is treated with 2,6-lutidine (30 mmol) and ^{t}butyldimethylsilyl triflate (25 mmol) for 1 hour. The mixture is poured into water and extracted with CH_2Cl_2. The extract is washed sequentially with 1 M phosphate buffer, pH 6, sat. $NaHCO_3$, and brine, then dried over $MgSO_4$, filtered, and evaporated. The product is isolated by silica gel chromatography.

EXAMPLE 20

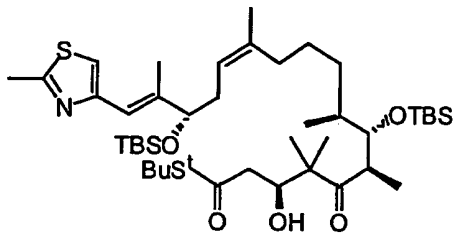
(*4R,5S,6S,13S*)-5-hydroxy-3-oxo-2,2,4,6,10,14-hexamethyl-13,15-di(^{t}butyldimethylsilyloxy)-15-(2-methyl-4-thiazolyl)pentadeca-10,14-dienal



A solution of N-methoxy-N-methyl (*4R,5S,6S,13S*)-5-hydroxy-3-oxo-2,2,4,6,10,14-hexamethyl-13,15-di(^{t}butyldimethylsilyloxy)-15-(2-methyl-4-thiazolyl)pentadeca-10,14-dienamide (10 mmol) in 20 mL of toluene is cooled to –78 °C, and a 1.5 M solution of diisobutylaluminum hydride in toluene (50 mmol) is added dropwise over a 45 minute period. Stirring is continued until complete conversion of starting material as determined by thin-layer chromatographic analysis. Ethyl acetate (10 mL) is added, and the mixture is allowed to warm to ambient temperature. A 100-mL portion of 1 N HCl is added, and the mixture is stirred an additional 1 hour before extracting with CH_2Cl_2. The extract is dried over $MgSO_4$, filtered, and evaporated. The product is purified by silica gel chromatography.

EXAMPLE 21

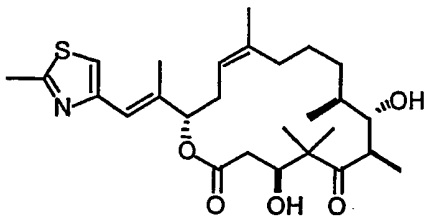
<u>^{t}Butyl (*3R,6R,7S,8S*)-3-hydroxy-5-oxo-4,4,6,8,12,16-hexamethyl-7,15-di(^{t}butyldimethylsilyloxy)-17-(2-methyl-4-thiazolyl)heptadeca-12,16-dienoic acid thioester</u>



A solution of ^{t}butyl thioacetate (10 mmol) in CH_2Cl_2 (10 mL) is cooled on ice and treated sequentially with (*2S,5S*)-2,5-dimethylborolane triflate (11 mmol) and diisopropylethylamine (12 mmol). The mixture is cooled to –78 °C, and a solution of (*4R,5S,6S,13S*)-5-hydroxy-3-oxo-2,2,4,6,10,14-hexamethyl-13,15-di(^{t}butyldimethylsilyloxy)-15-(2-methyl-4-thiazolyl)pentadeca-10,14-dienal (9 mmol) in 10 mL of CH_2Cl_2 is added dropwise. The mixture is stirred for 30 min, then warmed to 0 °C and kept for 1 hour. A mixture of 25 mL of 1 M phosphate buffer, pH 7, and 75 mL of methanol is added, followed by 75 mL of 2:1 methanol/50% H_2O_2, and the mixture is stirred for 1 hour at 0 °C. The reaction is concentrated to a slurry *in vacuo*, diluted with water, and extracted with ethyl acetate. The extract is washed sequentially with 5% $NaHCO_3$ and brine, then dried over $MgSO_4$, filtered, and evaporated. The product is purified by silica gel chromatography.

EXAMPLE 22

<u>Epothilone D</u>

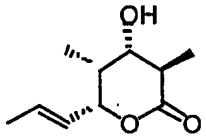


A solution of ^{t}butyl (*3R,6R,7S,8S*)-3-hydroxy-5-oxo-4,4,6,8,12,16-hexamethyl-7,15-di(^{t}butyldimethylsilyloxy)-17-(2-methyl-4-thiazolyl)heptadeca-12,16-dienoic acid thioester (1 mmol) in 20 mL of tetrahydrofuran is cooled on ice and treated with anhydrous tetrabutylammonium fluoride (5 mmol). Upon completion, the reaction is poured into water and extracted with CH_2Cl_2. The extract is washed sequentially with water, sat.

$NaHCO_3$, and brine. The extract is dried over $MgSO_4$, filtered, and evaporated. The product is purified by silica gel chromatography.

EXAMPLE 23

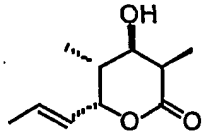
Preparation of *(2R,3S,4S,5R)*-3,5-dihydroxy-2,4-dimethyl-6-octenoic acid δ-lactone



(2R,3S,4S,5R)-3,5-dihydroxy-2,4-dimethyl-6-octenoic acid δ-lactone is made by providing 2-methyl-3-hydroxyl-4-hexenoiate N acetylcysteamine thioester to a functional PKS system comprising DEBS1 and a releasing domain wherein the ketosynthase domain of module 1 has been inactivated, fermented according to the procedure of Example 4.

EXAMPLE 24

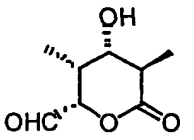
Preparation of *(2R,3R,4S,5R)*-3,5-dihydroxy-2,4-dimethyl-6-octenoic acid δ-lactone



(2R,3S,4S,5R)-3,5-dihydroxy-2,4-dimethyl-6-octenoic acid δ-lactone is made by providing 2-methyl-3-hydroxyl-4-hexenoiate N acetylcysteamine thioester to a functional PKS system comprising DEBS1 and a releasing domain wherein the ketosynthase domain of module 1 has been inactivated and where the ketoreductase ("KR") domain of module 2 of DEBS has been replaced with the ketoreductase domain of module 2 of rapamycin, fermented according to the procedure of Example 4.

EXAMPLE 25

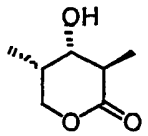
Preparation of *(2R,3S,4S,5S)*-3,5-dihydroxy-2,4-dimethyl-6-oxo-6-hexanoic acid δ-lactone



A solution of *(2R,3S,4S,5R)*-3,5-dihydroxy-2,4-dimethyl-6-octenoic acid δ-lactone (1.84 g) in 10 mL of CH_2Cl_2 is cooled to –78 °C and a stream of ozone is bubbled through until a blue color persists. The mixture is swept with a stream of nitrogen gas until the blue color dissipates, then treated with dimethylsulfide (2 mL) and warmed to ambient temperature and concentrated. The product is isolated by silica gel chromatography.

EXAMPLE 26

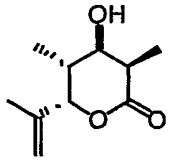
Preparation of *(2R,3S,4S)*-3,5-dihydroxy-2,4-dimethyl-6-pentanoic acid δ-lactone



A solution of *(2R,3S,4S,5S)*-3,5-dihydroxy-2,4-dimethyl-6-oxo-6-hexanoic acid δ-lactone (1.72 g) and tris(triphenylphosphine)rhodium chloride (9.25 g) in 100 mL of benzene is heated at reflux for 8 hours, then evaporated. The product is isolated by silica gel chromatography.

EXAMPLE 27

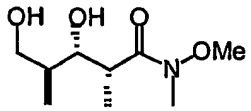
Preparation of *(2R,3R,4S,5R)*-3,5-dihydroxy-2,4,6-trimethyl-6-heptenoic acid δ-lactone



(2R,3R,4S,5R)-3,5-dihydroxy-2,4,6-trimethyl-6-heptenoic acid δ-lactone is made by providing 2,4-dimethyl-3-hydroxy-4-pentenoate N-acetylcysteamine thioester to a functional PKS system comprising DEBS1 and a releasing domain wherein the ketosynthase domain of module 1 has been inactivated and where the ketoreductase ("KR") domain of module 2 of DEBS has been replaced with the ketoreductase domain of module 2 of rapamycin, fermented according to the procedure of Example 4.

EXAMPLE 28

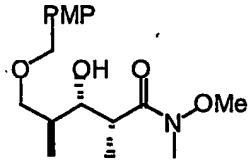
N-methoxy-N-methyl (*2R,3S,4S*)-3,5-dihydroxy-2,4-dimethylpentanamide



A solution of 2 M trimethylaluminum in toluene (10 mmol) is added dropwise to a suspension of N,O-dimethylhydroxylamine hydrochloride (10 mmol) in 8 mL of CH_2Cl_2 at 0 °C. The resulting homogeneous solution is stirred for 30 min at ambient temperature. A solution of (*2R,3S,4S*)-3,5-dihydroxy-2,4-dimethyl-6-pentanoic acid δ-lactone (2 mmol) in 4 mL of CH_2Cl_2 is added over 10 min, and the resulting solution is heated at reflux until complete consumption of starting material. Upon cooling, the mixture is poured into 1 M HCl, and extracted with CH_2Cl_2. The extract is washed sequentially with 5% $NaHCO_3$ and brine, dried over $MgSO_4$, filtered, and evaporated. The product is purified by silica gel chromatography.

EXAMPLE 29

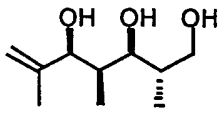
N-methoxy-N-methyl (*2S,3S,4S*)-3-hydroxy-2,4-dimethyl-5-((4-methoxybenzyl)oxy)pentanamide ("Common precursor")



A solution of N-methoxy-N-methyl (*2R,3S,4S*)-3,5-dihydroxy-2,4-dimethylpentanamide (1 mmol) in 5 mL of methanol is treated with dibutyltin oxide (250 mg) at reflux for 1 hour, then treated with 4-methoxybenzyl chloride (250 mg). The mixture is evaporated, and the product is isolated by chromatography on silica gel.

EXAMPLE 30

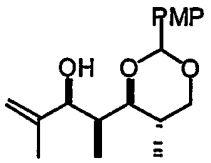
(2S,3S,4S,5R)-2,4,6-trimethylhept-6-en-1,3,5-triol



A solution of (2R,3R,4S,5R)-3,5-dihydroxy-2,4,6-trimethyl-6-heptenoic acid δ-lactone (1 mmol) in 1.5 mL of tetrahydrofuran is added dropwise to a suspension of lithium aluminum hydride (2 mmol) in 3 mL of tetrahydrofuran cooled on ice. After stirring for 1 hour, the mixture is warmed to ambient temperature and stirred for 24 hours. The reaction is then cooled on ice and treated sequentially with water and 15% KOH, then stirred vigorously for 24 hours at ambient temperature. The solids are removed by filtration, and the eluent is concentrated under vacuum. The product is purified by silica gel chromatography.

EXAMPLE 31

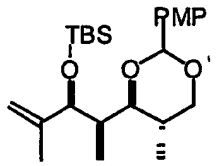
(2S,3S,4S,5R)-2,4,6-triimethylhept-6-en-1,3,5-triol 1,3-(4-methoxybenzylidene)acetal



A mixture of (2S,3S,4S,5R)-2,4,6-trimethylhept-6-en-1,3,5-triol (1 mmol) and 4-methoxybenzaldehyde dimethylacetal (1.2 mmol) in 5 mL of CH_2Cl_2 is treated with camphorsulfonic acid (0.05 mmol) for 12 hours. Saturated $NaHCO_3$ is added, and the mixture is extracted with CH_2Cl_2. The extract is washed with brine, dried over $NaSO_4$, filtered, and evaporated. The product is purified by silica gel chromatography.

EXAMPLE 32

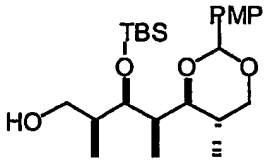
(2S,3S,4S,5R)-5-(tbutyldimethylsilyloxy)-2,4,6-triimethylhept-6-en-1,3-diol 1,3-(4-methoxybenzylidene)acetal



A mixture of (2S,3S,4S,5R)-2,4,6-triimethylhept-6-en-1,3,5-triol 1,3-(4-methoxybenzylidene)acetal (10 mmol), *tert*-butyldimethylsilyl chloride (12 mmol), and imidazole (20 mmol) in 25 mL of dimethylformamide is stirred for 24 hours at ambient temperature, then poured into water and extracted with ether. The extract is dried over $MgSO_4$, filtered, and evaporated. The product is purified by silica gel chromatography.

EXAMPLE 33

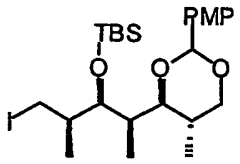
Alternate preparation of (2S,3S,4R,5S)- 2,4,6-trimethyl-5,7-dihydroxy-3-(tbutyldimethylsilyloxy)-1-heptanol 5,7-(4-methoxybenzylidene)acetal



A solution of (2S,3S,4S,5R)-5-(*tert*-butyldimethylsilyloxy)-2,4,6-triimethylhept-6-en-1,3-diol 1,3-(4-methoxybenzylidene)acetal, (10 mmol) and tris(triphenylphosphine)rhodium chloride (0.5 mmol) in 10 mL of tetrahydrofuran is treated with a 1 M solution of catecholborane in tetrahydrofuran (30 mmol) for 8 hours at ambient temperature. Ethanol (3 mL) is added followed by 25 mL of sat. $NaHCO_3$ and 10 mL of 30% H_2O_2. The mix is vigorously stirred for 2 hours, then diluted with sat. Na_2SO_3 and extracted with ether. The extract is washed with brine, dried over Na_2SO_4, filtered, and evaporated. The product is purified by silica gel chromatography.

EXAMPLE 34

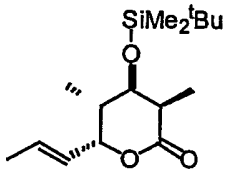
(2R,3S,4R,5S)- 2,4,6-trimethyl-5,7-dihydroxy-3-(tbutyldimethylsilyloxy)-1-iodoheptane 5,7-(4-methoxybenzylidene) acetal



A solution of (2S,3S,4R,5S)- 2,4,6-trimethyl-5,7-dihydroxy-3-(tbutyldimethylsilyloxy)-1-heptanol 5,7-(4-methoxybenzylidene) acetal (10 mmol) in 70 mL of 1:2 benzene/ether is treated with triphenylphosphine (15 mmol), imidazole (15 mmol), and iodine (15 mmol) with vigorous stirring. After 1 hour, the mix is diluted with ether and washed sequentially with sat. sodium thiosulfate and brine, dried over Na_2SO_4, filtered, and evaporated. The product is purified by silica gel chromatography.

EXAMPLE 35

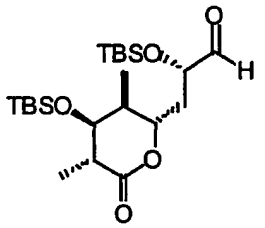
(2R,3R,4S,5R)-5-hydroxy-2,4-dimethyl-3-(*tert*-butyldimethylsilyloxy)-6-octenoic acid δ-lactone



A solution of (2R,3R,4S,5R)-3,5-dihydroxy-2,4-dimethyl-6-octenoic acid δ-lactone (10 mmol), 2,6-lutidine (15 mmol), and *tert*-butyldimethylsilyl triflate (11 mmol) in 10 mL of CH_2Cl_2 is stirred for 1 hour. The mixture is washed with water, then dried over $MgSO_4$, filtered, and evaporated. The product is purified by silica gel chromatography.

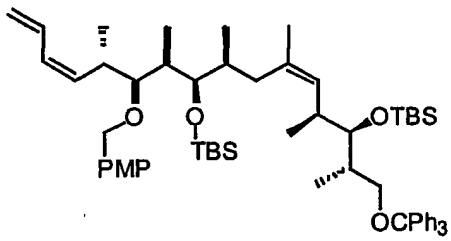
EXAMPLE 36

(*2R,3S,4S,5R,7S*)-5-hydroxy-8-oxo-2,4-dimethyl-3,7-di(*tert*-butyldimethylsilyloxy)-8-octanoic acid δ-lactone



A solution of (*2R,3S,4S,5R,7S*)-5-hydroxy-2,4,9-trimethyl-3,7-di(*tert*-butyldimethylsilyloxy)-8-decenoic acid δ-lactone (10 mmol) in 150 mL of CH_2Cl_2 is cooled to –78 °C, and a stream of ozone in oxygen is bubbled through until a blue color persists. The mixture is purged with a stream of air for 10 minutes, then triphenylphosphine (11 mmol) is slowly added. The mixture is allowed to warm to ambient temperature and stirred for 1 hour, then concentrated. The product is isolated by silica gel chromatography.

EXAMPLE 37

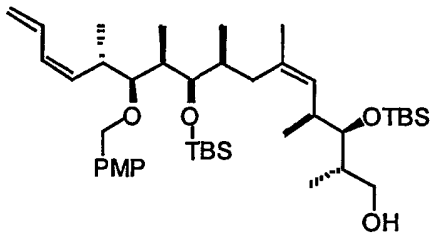


A solution of the product of Example 51 (10 mmol) in 100 mL of CH_2Cl_2 is treated with Dess-Martin periodinane (12 mmol) and $NaHCO_3$ (30 mmol) for 3 hours, then quenched by addition of sat. NaS_2O_3 and sat. $NaHCO_3$ solutions. The mixture is extracted with ether, and the extract is washed with water and brine, dried over $MgSO_4$, filtered, and evaporated. The crude aldehyde is used immediately in the next step.

A 1.7M solution of *tert*-butyllithium in pentane (20 mmol) is added to a –78 °C solution of allyldiphenylphosphine (20 mmol) in 60 mL of degassed tetrahydrofuran. The mixture is

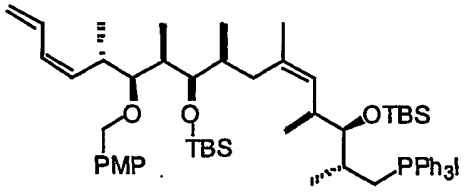
stirred for 5 min, then warmed to 0 °C, stirred for 30 min, and recooled to –78 °C. Titanium tetraisopropoxide (20 mmol) is added. After 30 min, a cold solution of the aldehyde from above in 35 mL of tetrahydrofuran is added and stirred for 1 hour. The solution is warmed to 0 °C, and methyl iodide (100 mL) is added. The solution is stirred at ambient temperature for 16 hours, quenched by addition of phosphate buffer, pH 7, and extracted with CH_2Cl_2. The extract is washed with brine, dried over $MgSO_4$, filtered, and evaporated. The product is purified by silica gel chromatography.

EXAMPLE 38



Methanol (0.75 mL) is added to a 0 °C solution of chlorocatecholborane (72.5 mmol) in CH_2Cl_2 (20 mL). The resulting solution is added dropwise to a solution of the product of Example 52 (10 mmol) in CH_2Cl_2 at 0 °C, and the reaction is monitored by thin-layer chromatography . Once the reaction is ca. 90% complete, the mixture is treated with sat. $NaHCO_3$ and stirred for 15 minutes. The mixture is extracted with ether, and the extract is washed with brine, dried with $MgSO_4$, filtered, and evaporated. The product is purified by silica gel chromatography.

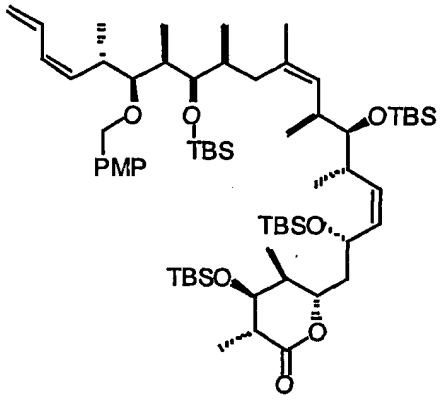
EXAMPLE 39



A solution of iodine (20 mmol) in 50 mL of ether is added dropwise to a solution of the alcohol product of Example 53 (10 mmol), triphenylphosphine (25 mmol), and imidazole (25 mmol) in 200 mL of 1:1 ether/benzene cooled to 0 °C. The resulting suspension is stirred for 30 min, then poured into 750 mL of 1:1 water/hexanes. The phases are separated, and the aqueous phase is extracted with hexanes. The organic phases are combined and ashed with sat. aq. $Na_2S_2O_3$, water, and brine, then dried over $MgSO_4$,

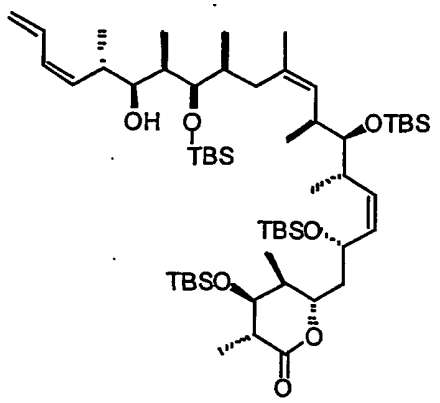
filtered, and evaporated to yield a slurry. The slurry is loaded onto a short column of silica gel with a small volume of CH_2Cl_2, and the product is rapidly eluted using a mixture of 2% ether + 0.05% Et_3N in hexanes. The eluent is concentrated in vacuo to yield the crude iodide. This is dissolved in 25 mL of 7:3 benzene/toluene, treated with 1 mL of diisopropylethylamine and 12.5 g of triphenylphosphine, and loaded into a high pressure apparatus and subjected to a pressure of 12.8 kbar for 14 days. The mixture is then concentrated and chromatographed on silica gel to provide the product, which is dried by repeated evaporated from benzene followed by heated under vacuum at 50 °C for 12 hours.

EXAMPLE 40



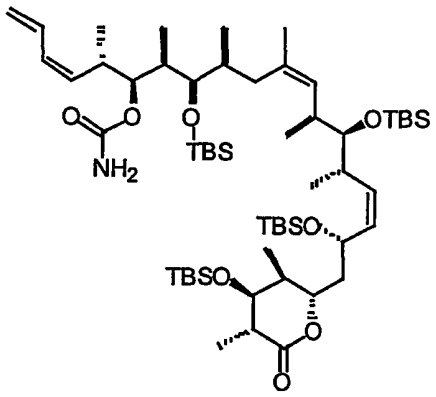
The phosphonium salt product of Example 54 (11 mmol) is dissolved in 60 mL of tetrahydrofuran, and is placed under argon atmosphere, and is cooled to –20 °C. A 1.0 M solution of sodium bis(trimethylsilyl)amide in tetrahydrofuran (10.5 mmol) is added, the mixture is stirred for 15 min, warmed to 0 °C, stirred for 30 min, then recooled to –25 °C. A solution of the aldehyde from Example 47 (11 mmol) in 30 mL of tetrahydrofuran is added over 15 minutes, then the mixture is warmed slowly to –10 °C and quenched with a mixture of sat. NH_4Cl and ether. The mixture is extracted with ether, and the extract is dried over $MgSO_4$, filtered, and evaporated. The product is isolated by silica gel chromatography.

EXAMPLE 41



A solution of the product of Example 55 (10 mmol) in 100 mL of CH_2Cl_2 is cooled to 0 °C and treated with water (5 mL) and 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (12 mmol) for 10 minutes. The mixture is warmed to ambient temperature, stirred for 5 minutes, treated with saturated $NaHCO_3$, and extracted with CH_2Cl_2. The extract is washed with water and brine, then dried over $MgSO_4$, filtered, and evaporated . The product is isolated by silica gel chromatography.

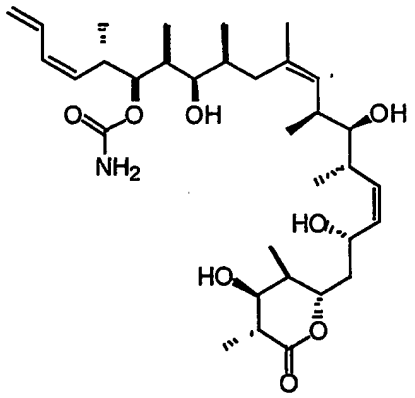
EXAMPLE 42



A solution of the alcohol from Example 56 (10 mmol) in CH_2Cl_2 (100 mL) is treated with trichloroacetylisocyanate (12 mmol) for 1 hour at ambient temperature. The solution is loaded onto neutral alumina, and eluted from the alumina after 4 hours using ethyl acetate. The eluent is concentrated, and the product is purified by silica gel chromatography.

EXAMPLE 43

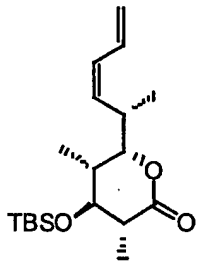
(+)-Discodermolide



The product of Example 57 (1 mmol) is dissolved in 300 mL of methanol and stirred for 15 minutes, then 3N HCl (200 mL) is added in 20-mL portions at such a rate so as to minimize precipitation. After completion of this addition, additional 3N HCl (100 mL) is added in 4 portions at 15 minute intervals. After 8 hours, a final portion of 3N HCl (100 mL) is added, the solution is stirred for 2 hours, and finally diluted with ethyl acetate (2000 mL). The phases are separated, and the aquoeus phase is extracted with ethyl acetate. The organic extracts are combined, washed with sat. $NaHCO_3$ and brine, dried with Na_2SO_4, filtered, and evaporated. The product is chromatographed on silica gel, then crystallized from acetonitrile.

EXAMPLE 44

(*7Z*)-(*2R,3S,4R,5S,6S*)-5-hydroxy-3-(ᵗbutyldimethylsilyloxy)-2,4,6-trimethyldeca-7,9-dienoate δ-lactone

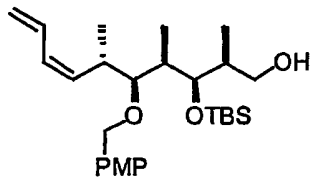


A solution of allyldiphenylphosphine (10.8 mmol) in 35 mL of tetrahydrofuran is cooled to –78 °C and treated with a 1.7 M solution of *tert*-butyllithium in pentane (10.8 mmol) for 5 minutes. The solution is warmed to 0 °C, stirred for an additional 30 minutes, then recooled to –78 °C. Titanium tetraisopropoxide (10.8 mmol) is added and stirring is continued for 30 minutes prior to addition of a –78 °C solution of (*2R,3S,4R,5S,6R*)-5-hydroxy-3-(ᵗbutyldimethylsilyloxy)-7-oxo-2,4,6-trimethylheptanoate δ-lactone (5.4 mmol)

in 20 mL of tetrahydrofuran. After 1 hour, the mixture is warmed to 0 °C, iodomethane (3.4 mL) is added, and the mixture is allowed to stir at ambient temperature for 16 hours.Phosphate buffer, pH 7.0, is added and the mixture is extracted with CH_2Cl_2. The extract is washed with brine, dried over $MgSO_4$, filtered, and evaporated. The product is purified by silica gel chromatography.

EXAMPLE 45

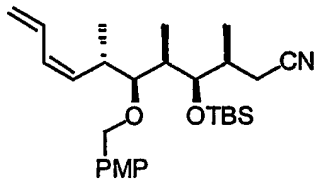
(7Z)-(2S,3R,4R,5S,6S)-5-((4-methoxybenzyl)oxy)-3-((tbutyldimethylsilyl)oxy)-2,4,6-trimethyldeca-7,9-diene-1-ol



A solution of 4-methoxybenzyl alcohol (11 mmol) in 10 mL of tetrahydrofuran is added dropwise to a suspension of sodium hydride (12 mmol) in 50 mL of tetrahydrofuran. After cessation of gas evolution, the mixture is treated with a solution of *(7Z)-(2R,3S,4R,5S,6S)*-5-hydroxy-3-(tbutyldimethylsilyloxy)-2,4,6-trimethyldeca-7,9-dienoate δ-lactone (10 mmol) in 10 mL of tetrahydrofuran. The reaction is monitored by thin-layer chromatography. Upon disappearance of the lactone, the mixture is treated with 4-methoxybenzyl bromide (12 mmol) and tetrabutylammonium iodide (1 mmol) for 5 hours at ambient temperature. The mixture is quenched by addition of sat. aq. NH_4Cl and extracted with ether. The extract is washed with brine, dried over $MgSO_4$, filtered, and evaporated to yield the crude protected ester. The crude ester is dissolved in 10 mL of tetrahydrofuran and added to a 1.0 M solution of lithium aluminum hydride (10 mL) at 0 °C. After stirring for 3 hours, the mixture is quenched with sat. aq. NH_4Cl and extracted with ether. The extract is washed with brine, dried over $MgSO_4$, filtered, and evaporated to yield the crude alcohol. The product is purified by silica gel chromatography.

EXAMPLE 46

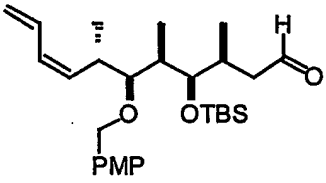
(7Z)-(2S,3R,4R,5S,6S)-5-((4-methoxybenzyl)oxy)-3-((tbutyldimethylsilyl)oxy)-2,4,6-trimethylundeca-7,9-dienenitrile



A solution of (7Z)-(2S,3R,4R,5S,6S)-5-((4-methoxybenzyl)oxy)-3-((tbutyldimethylsilyl)oxy)-2,4,6-trimethyldeca-7,9-diene-1-ol (10 mmol) in 70 mL of 1:2 benzene/ether is treated with triphenylphosphine (15 mmol), imidazole (15 mmol), and iodine (15 mmol) with vigorous stirring. After 1 hour, the mix is diluted with ether and washed sequentially with sat. sodium thiosulfate and brine, dried over Na_2SO_4, filtered, and evaporated. The crude iodide is dissolved in methylsulfoxide (50 mL) and treated with sodium cyanide (15 mmol). The solution is diluted with water and extracted with ether. The extract is washed with brine, dried over $MgSO_4$, filtered, and evaporated. The product is isolated by silica gel chromatography.

EXAMPLE 47

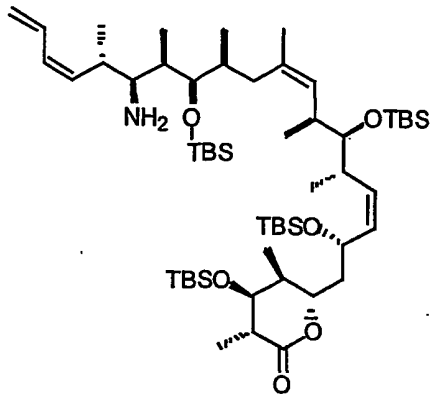
(7Z)-(2S,3R,4R,5S,6S)-5-((4-methoxybenzyl)oxy)-3-((tbutyldimethylsilyl)oxy)-2,4,6-trimethylundeca-7,9-dieneal



A solution of (7Z)-(2S,3R,4R,5S,6S)-5-((4-methoxybenzyl)oxy)-3-((tbutyldimethylsilyl)oxy)-2,4,6-trimethylundeca-7,9-dienenitrile (10 mmol) in tetrahydrofuran (10 mL) is cooled to –60 °C and treated with a 1.0 M solution of diisobutylaluminum hydride in toluene (12 mL). After 1 hour, the mix is allowed to warm to ambient temperature and kept an additional 3 hours before addition of sat. aq. NH_4Cl. After 30 minutes, the mixture is carefully acidified with 5% H_2SO_4 and immediately extracted with ether. The extract is washed with brine, dried over $MgSO_4$, filtered, and evaporated. The product is isolated by silica gel chromatography.

EXAMPLE 48

3,7,11,17-tetra-(O-*tert*-butyldimethylsilyl)-19-(O-descarbamoyloxy)-19-aminodiscodermolide

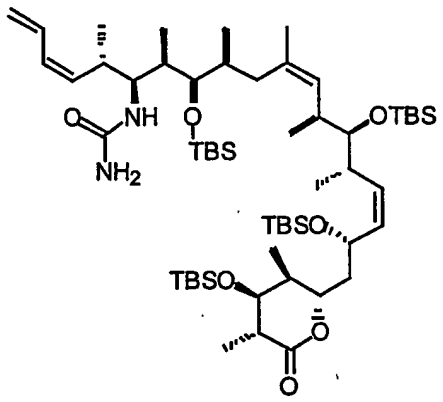


A solution of 3,7,11,17-tetra-(O-*tert*-butyldimethylsilyl)-19-(O-descarbamoyl)-19-epi-discodermolide (1 mmol) in 50 mL of DMF is cooled to 0 °C and treated with diisopropylethylamine (1.5 mmol) and methanesulfonic anhydride (1.5 mmol). After 1 hour, the mixture is treated with sodium azide (10 mmol). The reaction is continued for 2 hours at 50 °C, then poured into sat. $NaHCO_3$ and extracted with CH_2Cl_2. The extract is washed with brine, dried over $MgSO_4$, filtered, and evaporated. The 19-azido-product is purified by silica gel chromatography.

The 19-azide is dissolved in 10 mL of THF and treated with 5 mL of a 1 M solution of trimethylphosphine in THF. After 2 hours, water is added and the mixture is stirred overnight, then concentrated to dryness. The 19-amine is isolated by silica gel chromatography.

EXAMPLE 49

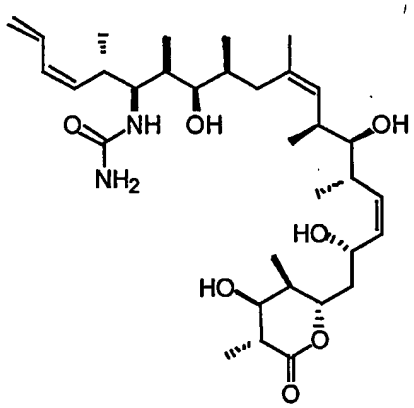
3,7,11,17-tetra-(O-*tert*-butyldimethylsilyl)-19-(O-descarbamoyloxy)-19-(carbamoylamino)discodermolide



A solution of 3,7,11,17-tetra-(O-*tert*-butyldimethylsilyl)-19-(O-descarbamoyloxy)-19-aminodiscodermolide (1 mmol) in CH_2Cl_2 (10 mL) is treated with trichloroacetylisocyanate (1.2 mmol) for 1 hour at ambient temperature. The solution is loaded onto neutral alumina, and eluted from the alumina after 4 hours using ethyl acetate. The eluent is concentrated, and the product is purified by silica gel chromatography.

EXAMPLE 50

19-(O-descarbamoyloxy)-19-(carbamoylamino)discodermolide

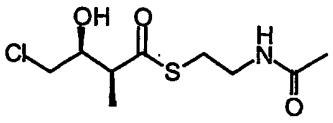


3,7,11,17-tetra-(O-*tert*-butyldimethylsilyl)-19-(O-descarbamoyloxy)-19-(carbamoylamino)discodermolide (1 mmol) is dissolved in 300 mL of methanol and stirred for 15 minutes, then 3N HCl (200 mL) is added in 20-mL portions at such a rate so as to minimize precipitation. After completion of this addition, additional 3N HCl (100 mL) is added in 4 portions at 15 minute intervals. After 8 hours, a final portion of 3N HCl (100

mL) is added, the solution is stirred for 2 hours, and finally diluted with ethyl acetate (2000 mL). The phases are separated, and the aquoeus phase is extracted with ethyl acetate. The organic extracts are combined, washed with sat. $NaHCO_3$ and brine, dried with Na_2SO_4, filtered, and evaporated. The product is chromatographed on silica gel.

EXAMPLE 51

(±)-(2S*,3R*)-4-chloro-3-hydroxy-2-methylbutyrate N-acetylcysteamine thioester



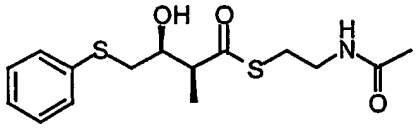
Step 1. A solution of N-propionyl-2-benzoxazolone (100.0 g) in anhydrous CH_2Cl_2 (1100 mL) is cooled to 3 °C with mechanical stirring under N_2 atmosphere. $TiCl_4$ (58.4 mL) is added at a rate such that the internal temperature remains below 10 °C (ca. 10 minutes). The resulting yellow slurry is stirred vigorously for 40 minutes, then triethylamine (87.4 mL) is added at a rate such that the internal temperature remains below 10 °C (ca. 10 minutes). The resulting deep red solution is stirred for 80 minutes. A 1 M solution of chloroacetaldehyde in CH_2Cl_2 (1000 mL) is added at a rate such that the internal temperature remains below 10 °C (ca. 20 minutes), and the reaction is followed by thin-layer chromatography (4:1 hexanes/ethyl acetate). After stirring for 90 minutes, the reaction is quenched by addition of 450 mL of 2 N HCl. The phases are separated, and the organic phase is filtered through a pad of silica gel. The silica gel is washed with ether, and the combined organic are concentrated under vacuum to a. The product is collected by vacuum filtration and rinsed with hexanes to yield a colorless solid.

Step 2. One molar equivalent of sodium methoxide (25% w/v in methanol; ca. 150 mL) is added in a slow stream to a solution of N,S-diacetylcysteamine (173 g) in methanol (910 mL) under N_2. When half of the calculated volume has been added, the reaction is monitored by TLC (1:1 ethyl acetate/hexanes), and methoxide addition is continued until complete conversion of the N,S-diacetylcysteamine to N-acetylcysteamine. Acetic acid (50 g) is added, and the resulting solution of sodium thiolate is cannulated into a flask containing solid (±)-N-[*syn*-4-chloro-3-hydroxy-4-butanoyl]-2-benzoxazolone (270 g) under N_2. After 15 minutes, the reaction is quenched with solid oxalic acid dihydrate (80.4 g), filtered, and concentrated to a yellow oil. The residue is dissolved in 2:1 hexanes/ethyl

acetate and submitted to batch elution chromatography on SiO_2. The silica is washed with 2:1 hexanes/ethyl acetate to remove 2-benzoxazolone, then with ethyl acetate/methanol (9:1) to elute the product thioester. Evaporation of the thioester-containing eluent yields the product.

EXAMPLE 52

(±)-(2S*,3R*)-3-hydroxy-2-methyl-4-(phenylthio)butyrate N-acetylcysteamine thioester



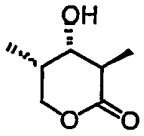
Step 1. A solution of N-propionyl-2-benzoxazolone (100.0 g) in anhydrous CH_2Cl_2 (1100 mL) is cooled to 3 °C with mechanical stirring under N_2 atmosphere. $TiCl_4$ (58.4 mL) is added at a rate such that the internal temperature remains below 10 °C (ca. 10 minutes). The resulting yellow slurry is stirred vigorously for 40 minutes, then triethylamine (87.4 mL) is added at a rate such that the internal temperature remains below 10 °C (ca. 10 minutes). The resulting deep red solution is stirred for 80 minutes. Phenylthioacetaldehyde (160 gm) is added at a rate such that the internal temperature remains below 10 °C (ca. 20 minutes), and the reaction is followed by thin-layer chromatography (4:1 hexanes/ethyl acetate). After stirring for 90 minutes, the reaction is quenched by addition of 450 mL of 2 N HCl. The phases are separated, and the organic phase is filtered through a pad of silica gel. The silica gel is washed with ether, and the combined organic are concentrated under vacuum to a. The product is collected by vacuum filtration and rinsed with hexanes to yield a colorless solid.

Step 2. One molar equivalent of sodium methoxide (25% w/v in methanol; ca. 150 mL) is added in a slow stream to a solution of N,S-diacetylcysteamine (173 g) in methanol (910 mL) under N_2. When half of the calculated volume has been added, the reaction is monitored by TLC (1:1 ethyl acetate/hexanes), and methoxide addition is continued until complete conversion of the N,S-diacetylcysteamine to N-acetylcysteamine. Acetic acid (50 g) is added, and the resulting solution of sodium thiolate is cannulated into a flask

containing solid (±)-N-[*syn*-4-phenylthio-3-hydroxy-4-butanoyl]-2-benzoxazolone (340 g) under N_2. After 15 minutes, the reaction is quenched with solid oxalic acid dihydrate (80.4 g), filtered, and concentrated to a yellow oil. The residue is dissolved in 2:1 hexanes/ethyl acetate and submitted to batch elution chromatography on SiO_2. The silica is washed with 2:1 hexanes/ethyl acetate to remove 2-benzoxazolone, then with ethyl acetate/methanol (9:1) to elute the product thioester. Evaporation of the thioester-containing eluent yields the product.

EXAMPLE 53

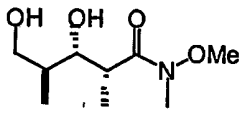
Alternate Preparation of *(2R,3S,4S)*-3,5-dihydroxy-2,4-dimethyl-6-pentanoic acid δ-lactone



A suspension of *(2R,3S,4S,5R)*-3,5-dihydroxy-2,4-dimethyl-6-octenoic acid δ-lactone (1.84 g) in 10 mL of water is treated with 15 mL of 1 N sodium hydroxide and stirred until complete dissolution is obtained. The pH of the solution is adjusted to 7.0, and a 4% solution of osmium tetraoxide in water (2 mL) is added followed by sodium periodate (10 g). The mixture is stirred vigorously overnight, then treated with sodium borohydride until disappearance of aldehyde as determined by reaction of an aliquot with acidic dinitrophenylhydrazine solution. The mixture is adjusted to pH 3 and extracted with ethyl acetate. The extract is dried over $MgSO_4$, filtered, and evaporated to yield the product.

EXAMPLE 54

N-methoxy-N-methyl *(2R,3S,4S)*-3,5-dihydroxy-2,4-dimethylpentanamide

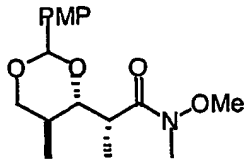


A solution of 2 M trimethylaluminum in toluene (10 mmol) is added dropwise to a suspension of N,O-dimethylhydroxylamine hydrochloride (10 mmol) in 8 mL of CH_2Cl_2 at 0 °C. The resulting homogeneous solution is stirred for 30 min at ambient temperature. A solution of *(2R,3S,4S)*-3,5-dihydroxy-2,4-dimethyl-6-pentanoic acid δ-lactone (2 mmol) in 4 mL of CH_2Cl_2 is added over 10 min, and the resulting solution is heated at reflux until complete consumption of starting material. Upon cooling, the mixture is poured into 1 M HCl, and extracted with CH_2Cl_2. The extract is washed sequentially with 5% $NaHCO_3$ and

brine, dried over $MgSO_4$, filtered, and evaporated. The product is purified by silica gel chromatography.

EXAMPLE 55

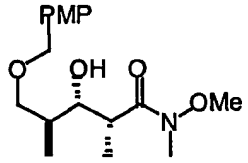
N-methoxy-N-methyl (*2R,3S,4S*)-3,5-dihydroxy-2,4-dimethylpentanamide 3,5-(4-methoxybenzylidene)acetal



A solution of N-methoxy-N-methyl (*2R,3S,4S*)-3,5-dihydroxy-2,4-dimethylpentanamide (10 mmol) and 4-methoxybenzaldehyde dimethylacetal (12 mmol) in 50 mL of CH_2Cl_2 is treated with anhydrous zinc chloride (1 mmol) for 20 hours. Saturated $NaHCO_3$ is added, and the mixture is extracted with CH_2Cl_2. The extract is washed with brine, dried over $NaSO_4$, filtered, and evaporated. The product is purified by silica gel chromatography.

EXAMPLE 56

Alternate preparation of N-methoxy-N-methyl (*2S,3S,4S*)-3-hydroxy-2,4-dimethyl-5-((4-methoxybenzyl)oxy)pentanamide

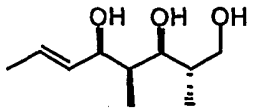


A solution of N-methoxy-N-methyl (*2R,3S,4S*)-3,5-dihydroxy-2,4-dimethylpentanamide 3,5-(4-methoxybenzylidene)acetal (1 mmol) in 5 mL of tetrahydrofuran is treated with 1.0 M HCl in ether and sodium cyanoborohydride. See e.g., Garegg & Hultberg, Carbohydrate Res. 1981, 93:123.

When complete, the reaction is quenched by addition of sat. $NaHCO_3$ and extracted with ether. The extract is washed sequentially with 1 N HCl, sat. $NaHCO_3$, and brine, then dried over $MgSO_4$, filtered, and evaporated. The product is purified by silica gel chromatography.

EXAMPLE 57

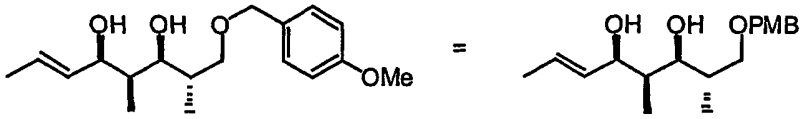
(2S,3S,4S,5R)-2,4-dimethyloct-6-en-1,3,5-triol



A solution of (2R,3R,4S,5R)-3,5-dihydroxy-2,4-dimethyl-6-octenoic acid δ-lactone (1 mmol) in 1.5 mL of tetrahydrofuran is added dropwise to a suspension of lithium aluminum hydride (2 mmol) in 3 mL of tetrahydrofuran cooled on ice. After stirring for 1 hour, the mixture is warmed to ambient temperature and stirred for 24 hours. The reaction is then cooled on ice and treated sequentially with water and 15% KOH, then stirred vigorously for 24 hours at ambient temperature. The solids are removed by filtration, and the eluent is concentrated under vacuum. The product is purified by silica gel chromatography.

EXAMPLE 58

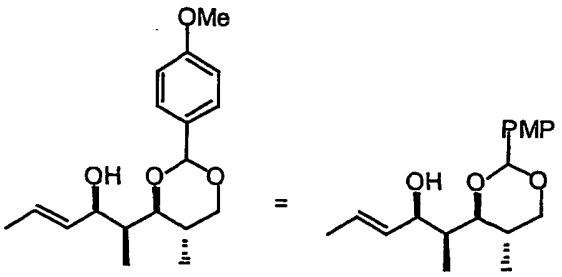
(2S,3S,4S,5R)-3,5-dihydroxy-2,4-dimethyl-1-((4-methoxybenzyl)oxy)oct-6-ene



A solution of (2S,3S,4S,5R-2,4-dimethyloct-6-en-1,3,5-triol (10 mmol) and freshly prepared 4-methoxybenzyl 2,2,2-trichloroacetimidate (13 mmol) in 15 mL of 1:2 CH_2Cl_2/cyclohexane is treated with pyridinium p-toluenesulfonate (0.5 mmol) at 0 °C. After 3 h, the mix is warmed to ambient temperature and stirred for an additional 48 hours. The solvent is evaporated, and the product is purified by silica gel chromatography.

EXAMPLE 59

(2S,3S,4S,5R)-2,4-dimethyloct-6-en-1,3,5-triol 1,3-(4-methoxybenzylidene) acetal

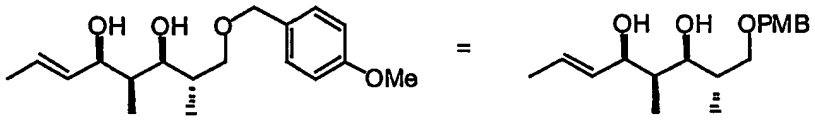


A mixture of (*2S,3S,4S,5R*)-2,4-dimethyloct-6-en-1,3,5-triol (1 mmol) and 4-methoxybenzaldehyde dimethylacetal (1.2 mmol) in 5 mL of CH_2Cl_2 is treated with camphorsulfonic acid (0.05 mmol) for 12 hours. Saturated $NaHCO_3$ is added, and the mixture is extracted with CH_2Cl_2. The extract is washed with brine, dried over $NaSO_4$, filtered, and evaporated. The product is purified by silica gel chromatography.

EXAMPLE 60

(*2S,3S,4S,5R*)-3,5-dihydroxy-2,4-dimethyl-1-((4-methoxybenzyl)oxy)oct-6-ene

Alternate preparation

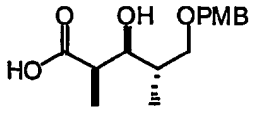


A solution of (*2S,3S,4S,5R*)-3,5-dihydroxy-2,4-dimethyloct-6-en-1-ol 1,3-(4-methoxyphenyl)acetal (1 mmol) in 5 mL of tetrahydrofuran is treated with 1.0 M HCl in ether and sodium cyanoborohydride. See, Garegg & Hultberg, Carbohydrate Res. 1981, 93:123.

When complete, the reaction is quenched by addition of sat. $NaHCO_3$ and extracted with ether. The extract is washed sequentially with 1 N HCl, sat. $NaHCO_3$, and brine, then dried over $MgSO_4$, filtered, and evaporated. The product is purified by silica gel chromatography.

EXAMPLE 61

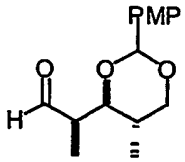
(*2R,3S,4S*)- 2,4-dimethyl-3-hydroxy-5-((4-methoxybenzyl)oxy)pentanoic acid



A solution of (*2S,3S,4S,5R*)-3,5-dihydroxy-2,4-dimethyl-1-((4-methoxybenzyl)oxy)oct-6-ene (1 mmol) in CCl_4 and acetonitrile is treated with sodium periodate in the presence of catalytic ruthenium dichloride. See, Webster et al., J. Org. Chem. 1987, 52:689-91.

EXAMPLE 62

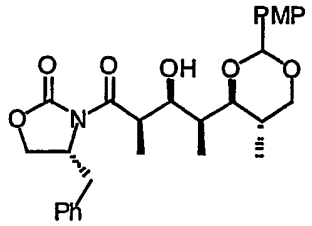
(*2R,3S,4S*)- 2,4-dimethyl-3,5-dihydroxypentanal 3,5-(4-methoxybenzylidene) acetal



A solution of (*2S,3S,4S,5R*)-2,4-dimethyloct-6-en-1,3,5-triol 1,3-(4-methoxybenzylidene) acetal (2.6 mmol) in a mix of 2,2-dimethylpropanol (30 mL), acetone (65 mL), and water (16 mL) is treated with osmium tetraoxide (0.08 mmol) and N-methylmorpholine N-oxide (13 mmol) for 11 hours at ambient temperature. The solution is diluted with 1 M NaH_2PO_4 and extracted with CH_2Cl_2. The extract is dried over Na_2SO_4, filtered, and evaporated to yield a crude triol intermediate. This material is dissolved in a mix of tetrahydrofuran (120 mL) and water (25 mL), and sodium metaperiodate (13 mmol) is added. After stirring vigorously for 24 hours, the mix is diluted with sat. $NaHCO_3$ and extracted with CH_2Cl_2. The extract is dried over Na_2SO_4, filtered, and evaporated. The product is purified by silica gel chromatography.

EXAMPLE 63

(*4R*)-4-benzyl-3-[(*2R,3S,4S,5S*)- 2,4,6-trimethyl-3,5,7-trihydroxyheptanoyl 5,7-(4-methoxybenzylidene) acetal]-2-oxazolidinone

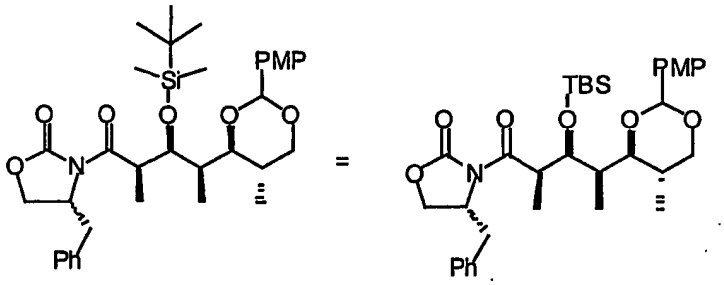


A solution of (*4R*)-4-benzyl-3-propionyl-2-oxazolidinone (10 mmol) in 10 mL of CH_2Cl_2 is cooled on ice and treated with 1.0 M solution of dibutylboron triflate in CH_2Cl_2 (11 mmol) followed by triethylamine (12 mmol). After stirring for 30 min, the solution is cooled to –78 °C and a solution of (*2R,3S,4S*)- 2,4-dimethyl-3,5-dihydroxypentanal 3,5-(4-methoxybenzylidene) acetal (9 mmol) in 10 mL of CH_2Cl_2 is added dropwise. The mixture is stirred for 30 min, then warmed to 0 °C and kept for 1 hour. A mixture of 25 mL of 1 M phosphate buffer, pH 7, and 75 mL of methanol is added, followed by 75 mL of 2:1 methanol/50% H_2O_2, and the mixture is stirred for 1 hour at 0 °C. The reaction is concentrated to a slurry *in vacuo*, diluted with water, and extracted with ethyl acetate. The

extract is washed sequentially with 5% $NaHCO_3$ and brine, then dried over $MgSO_4$, filtered, and evaporated. The product is purified by silica gel chromatography.

EXAMPLE 64

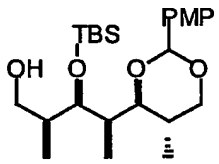
(*4R*)-4-benzyl-3-[(*2R,3S,4R,5S*)- 2,4,6-trimethyl-5,7-dihydroxy-3-(ᵗbutyldimethylsilyloxy)heptanoyl 5,7-(4-methoxybenzylidene) acetal]-2-oxazolidinone



A solution of (*4R*)-4-benzyl-3-[(*2R,3S,4S,5S*)- 2,4,6-trimethyl-3,5,7-trihydroxyheptanal 5,7-(4-methoxybenzylidene) acetal]-2-oxazolidinone (10 mmol) in 10 mL of CH_2Cl_2 is treated with ᵗbutyldimethylsilyl triflate (12 mmol) and 2,6-lutidine (20 mmol). After 1 hour, the mixture is poured into sat. $NaHCO_3$ and extracted with CH_2Cl_2. The extract is washed with brine, dried over Na_2SO_4, filtered, and evaporated. The product is purified by silica gel chromatography.

EXAMPLE 65

Alternate Preparation of (*2S,3S,4R,5S*)- 2,4,6-trimethyl-5,7-dihydroxy-3-(ᵗbutyldimethylsilyloxy)-1-heptanol 5,7-(4-methoxybenzylidene) acetal

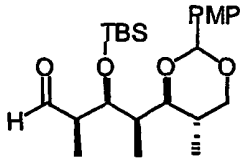


A solution of (*4R*)-4-benzyl-3-[(*2R,3S,4R,5S*)- 2,4,6-trimethyl-5,7-dihydroxy-3-(ᵗbutyldimethylsilyloxy)heptanoyl 5,7-(4-methoxybenzylidene) acetal]-2-oxazolidinone (10 mmol) in tetrahydrofuran (100 mL) is cooled to –30 °C, and ethanol (20 mmol) was added followed by addition of a 2 M solution of lithium borohydride in tetrahydrofuran (20 mmol) over 15 min. After stirring for 1 hour on ice, the mix is warmed to ambient temperature and stirred an additional 12 hours. The mix is diluted with ether and 40 mL of 1 N NaOH is added. After 2 hours, the phases are separated and the organic phase is

washed with brine, dried with Na_2SO_4, filtered, and evaporated. The product is purified by silica gel chromatography.

EXAMPLE 66

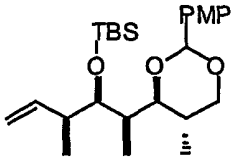
(2S,3S,4R,5S)- 2,4,6-trimethyl-5,7-dihydroxy-3-(^{t}butyldimethylsilyloxy)heptanal 5,7-(4-methoxybenzylidene) acetal



Oxalyl chloride (26 mmol) is added over 1 hour to a solution of methylsulfoxide (56 mmol) in CH_2Cl_2 (100 mL) cooled to –78 °C. After an additional 15 minutes, a –78 °C solution of *(2S,3S,4R,5S)*- 2,4,6-trimethyl-5,7-dihydroxy-3-(^{t}butyldimethylsilyloxy)-1-heptanol 5,7-(4-methoxybenzylidene) acetal (18 mmol) in CH_2Cl_2 (5 mL) is added over 15 min. After stirring for 30 min, diisopropylethylamine (86 mmol) is added over 15 min. The reaction is stirred an additional 30 min at –78 °C, then allowed to warm to ambient temperature over 1 hour. After addition of 1 N $NaHSO_4$, the mix is diluted with ether, washed with water, dried with $MgSO_4$, filtered, and evaporated. The product is purified by silica gel chromatography.

EXAMPLE 67

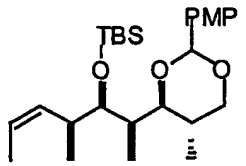
(3S,4R,5R,6S)- 3,5,7-trimethyl-6,8-dihydroxy-4-(^{t}butyldimethylsilyloxy)oct-1-ene 6,8-(4-methoxybenzylidene) acetal



A 1.6 M solution of butyllithium in hexane (12 mmol) is added dropwise to a suspension of methyltriphenylphosphonium bromide (12 mmol) in tetrahydrofuran. After 1 hour, a solution of *(2S,3S,4R,5S)*- 2,4,6-trimethyl-5,7-dihydroxy-3-(^{t}butyldimethylsilyloxy)-heptanal 5,7-(4-methoxybenzylidene) acetal (10 mmol) is added and stirred for 12 hours. The mixture is cooled to ambient temperature and concentrated. The residue is dissolved in 1:1 ether/hexane, filtered, and concentrated. The product is purified by silica gel chromatography.

EXAMPLE 68

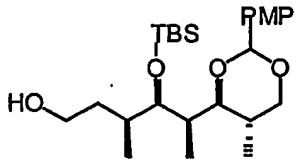
(4S,5R,6R,7S)- 4,6,8-trimethyl-7,9-dihydroxy-5-(^{t}butyldimethylsilyloxy)non-2-ene 7,9-(4-methoxybenzylidene) acetal



A 1.6 M solution of butyllithium in hexane (12 mmol) is added dropwise to a suspension of ethyltriphenylphosphonium bromide (12 mmol) in tetrahydrofuran. After 1 hour, a solution of *(2S,3S,4R,5S)*- 2,4,6-trimethyl-5,7-dihydroxy-3-(^{t}butyldimethylsilyloxy)-heptanal 5,7-(4-methoxybenzylidene) acetal (10 mmol) is added and stirred for 12 hours. The mixture is cooled to ambient temperature and concentrated. The residue is dissolved in 1:1 ether/hexane, filtered, and concentrated. The product is purified by silica gel chromatography.

EXAMPLE 69

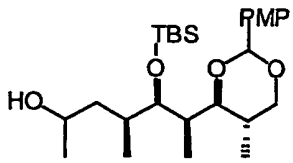
(3S,4R,5R,6S)- 3,5,7-trimethyl-6,8-dihydroxy-4-(^{t}butyldimethylsilyloxy)-1-octanol 6,8-(4-methoxybenzylidene)acetal



Borane-dimethylsulfide (23 mmol) is added to a solution of 2-methyl-2-butene (45 mmol) in tetrahydrofuran (60 mL) at 0 °C, and the solution is stirred for 2 hours. This solution of disiamylborane is added to a solution of *(3S,4R,5R,6S)*- 3,5,7-trimethyl-6,8-dihydroxy-4-(^{t}butyldimethylsilyloxy)oct-1-ene 6,8-(4-methoxybenzylidene) acetal (7.5 mmol) in 90 mL of tetrahydrofuran at 0 °C. After stirring for 90 minutes, ethanol (3 mL) is added followed by 25 mL of sat. $NaHCO_3$ and 10 mL of 30% H_2O_2. The mix is warmed to ambient temperature with vigorous stirring over 2 hours, then diluted with sat. Na_2SO_3 and extracted with ether. The extract is washed with brine, dried over Na_2SO_4, filtered, and evaporated. The product is purified by silica gel chromatography.

EXAMPLE 70

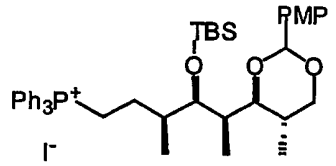
(4S,5R,6R,7S)- 4,6,8-trimethyl-7,9-dihydroxy-5-(t-butyldimethylsilyloxy)nonan-2-ol 7,9-(4-methoxybenzylidene) acetal



Borane-dimethylsulfide (23 mmol) is added to a solution of 2-methyl-2-butene (45 mmol) in tetrahydrofuran (60 mL) at 0 °C, and the solution is stirred for 2 hours. This solution of disiamylborane is added to a solution of (4S,5R,6R,7S)- 4,6,8-trimethyl-7,9-dihydroxy-5-(t-butyldimethylsilyloxy)non-2-ene 7,9-(4-methoxybenzylidene) acetal (7.5 mmol) in 90 mL of tetrahydrofuran at 0 °C. After stirring for 90 minutes, ethanol (3 mL) is added followed by 25 mL of sat. $NaHCO_3$ and 10 mL of 30% H_2O_2. The mix is warmed to ambient temperature with vigorous stirring over 2 hours, then diluted with sat. Na_2SO_3 and extracted with ether. The extract is washed with brine, dried over Na_2SO_4, filtered, and evaporated. The product is purified by silica gel chromatography.

EXAMPLE 71

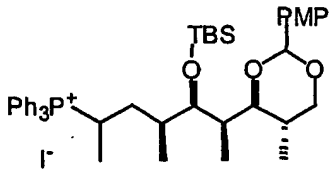
(3S,4R,5R,6S)-1-triphenylphosphonium-3,5,7-trimethyl-6,8-dihydroxy-4-(t-butyldimethylsilyloxy)octane 6,8-(4-methoxybenzylidene)acetal iodide



A solution of (3S,4R,5R,6S)- 3,5,7-trimethyl-6,8-dihydroxy-4-(t-butyldimethylsilyloxy)-1-octanol 6,8-(4-methoxybenzylidene)acetal (10 mmol) in 70 mL of 1:2 benzene/ether is treated with triphenylphosphine (15 mmol), imidazole (15 mmol), and iodine (15 mmol) with vigorous stirring. After 1 hour, the mix is diluted with ether and washed sequentially with sat. sodium thiosulfate and brine, dried over Na_2SO_4, filtered, and evaporated. The crude iodide is dissolved in tetrahydrofuran (50 mL) and treated with triphenylphosphine (15 mmol) at reflux. The solution is cooled to ambient temperature, and hexane is added to crystallize the phosphonium salt.

EXAMPLE 72

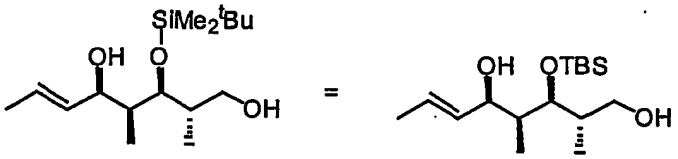
(*4S,5R,6R,7S*)-2-triphenylphosphonium-4,6,8-trimethyl-7,9-dihydroxy-5-(^{t}butyldimethylsilyloxy)nonan-2-ol 7,9-(4-methoxybenzylidene)acetal iodide



A solution of (*4S,5R,6R,7S*)- 4,6,8-trimethyl-7,9-dihydroxy-5-(^{t}butyldimethylsilyloxy)-nonan-2-ol 7,9-(4-methoxybenzylidene) acetal (10 mmol) in 70 mL of 1:2 benzene/ether is treated with triphenylphosphine (15 mmol), imidazole (15 mmol), and iodine (15 mmol) with vigorous stirring. After 1 hour, the mix is diluted with ether and washed sequentially with sat. sodium thiosulfate and brine, dried over Na_2SO_4, filtered, and evaporated. The crude iodide is dissolved in tetrahydrofuran (50 mL) and treated with triphenylphosphine (15 mmol) at reflux. The solution is cooled to ambient temperature, and hexane is added to crystallize the phosphonium salt.

EXAMPLE 73

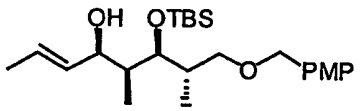
(*2R,3S,4S,5R*)-2,4-dimethyl-3-(*tert*-butyldimethylsilyloxy)oct-6-en-1,5-diol



A solution of (*2R,3R,4S,5R*)-5-hydroxy-2,4-dimethyl-3-(*tert*-butyldimethylsilyloxy)-6-octenoic acid δ-lactone (1 mmol) in 1.5 mL of tetrahydrofuran is added dropwise to a suspension of lithium aluminum hydride (2 mmol) in 3 mL of tetrahydrofuran cooled on ice. After stirring for 1 hour, the mixture is warmed to ambient temperature and stirred for 24 hours. The reaction is then cooled on ice and treated sequentially with water and 15% KOH, then stirred vigorously for 24 hours at ambient temperature. The solids are removed by filtration, and the eluent is concentrated under vacuum. The product is purified by silica gel chromatography.

EXAMPLE 74

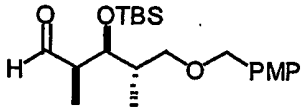
(2R,3R,4S,5R)-1-((4-methoxyphenyl)methoxy)-2,4-dimethyl-3-(*tert*-butyldimethylsilyloxy)oct-6-en-5-ol



A solution of *(2R,3S,4S,5R)*-2,4-dimethyl-3-(*tert*-butyldimethylsilyloxy)oct-6-en-1,5-diol (10 mmol) and freshly prepared 4-methoxybenzyl 2,2,2-trichloroacetimidate (13 mmol) in 15 mL of 1:2 CH_2Cl_2/cyclohexane is treated with pyridinium p-toluenesulfonate (0.5 mmol) at 0 °C. After 3 h, the mix is warmed to ambient temperature and stirred for an additional 48 hours. The solvent is evaporated, and the product is purified by silica gel chromatography.

EXAMPLE 75

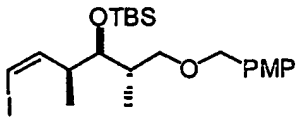
(2R,3S,4S)-5-((4-methoxyphenyl)methoxy)-2,4-dimethyl-3-(*tert*-butyldimethylsilyloxy)pentanal



A solution of *(2R,3R,4S,5R)*-1-((4-methoxyphenyl)methoxy)-2,4-dimethyl-3-(*tert*-butyldimethylsilyloxy)oct-6-en-5-ol (2.6 mmol) in a mix of 2,2-dimethylpropanol (30 mL), acetone (65 mL), and water (16 mL) is treated with osmium tetraoxide (0.08 mmol) and N-methylmorpholine N-oxide (13 mmol) for 11 hours at ambient temperature. The solution is diluted with 1 M NaH_2PO_4 and extracted with CH_2Cl_2. The extract is dried over Na_2SO_4, filtered, and evaporated to yield a crude triol intermediate. This material is dissolved in a mix of tetrahydrofuran (120 mL) and water (25 mL), and sodium metaperiodate (13 mmol) is added. After stirring vigorously for 24 hours, the mix is diluted with sat. $NaHCO_3$ and extracted with CH_2Cl_2. The extract is dried over Na_2SO_4, filtered, and evaporated. The product is purified by silica gel chromatography.

EXAMPLE 76

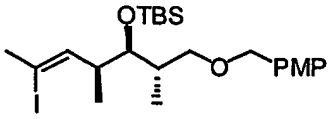
(3S,4S,5S)-1-iodo-6-((4-methoxyphenyl)methoxy)-3,5-dimethyl-4-(*tert*-butyldimethylsilyloxy)-1-hexene



A 1.6 M solution of butyllithium in hexanes (37 mmol) is added to a suspension of methyltriphenylphosphonium iodide (36 mmol) in tetrahydrofuran (200 mL) over 10 minutes. After an additional 10 minutes, the solution is added over 15 minutes to a –78 °C solution of iodine (32 mmol) in 300 mL of tetrahydrofuran. The resulting yellow slurry is stirred for 5 min, then warmed to –23 °C and kept for 10 minutes. A 1 M solution of sodium hexamethyldisilazide in tetrahydrofuran (31 mmol) is added over 8 minutes, and the mix is stirred an additional 15 minutes prior to addition of a solution of *(2R,3S,4S)*-5-((4-methoxyphenyl)methoxy)-2,4-dimethyl-3-(*tert*-butyldimethylsilyloxy)-pentanal (18 mmol) in 50 mL of tetrahydrofuran. After 10 min, the mix is warmed to ambient temperature and stirred for 3 hours. Methanol (10 mL) is added, and the mixture is concentrated under vacuum and the residue filtered through a pad of silica gel using 1:1 ethyl acetate/hexanes. The filtrate is washed sequentially with sat. $Na_2S_2O_3$ and brine, dried over $MgSO_4$, filtered, and concentrated. The product is purified by silica gel chromatography.

EXAMPLE 77

(4S,5S,6S)-2-iodo-7-((4-methoxyphenyl)methoxy)-4,6-dimethyl-5-(*tert*-butyldimethylsilyloxy)-2-heptene

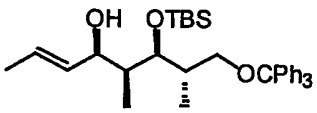


A 1.6 M solution of butyllithium in hexanes (37 mmol) is added to a suspension of ethyltriphenylphosphonium iodide (36 mmol) in tetrahydrofuran (200 mL) over 10 minutes. After an additional 10 minutes, the solution is added over 15 minutes to a –78 °C solution of iodine (32 mmol) in 300 mL of tetrahydrofuran. The resulting yellow slurry is stirred for 5 min, then warmed to –23 °C and kept for 10 minutes. A 1 M solution of sodium hexamethyldisilazide in tetrahydrofuran (31 mmol) is added over 8 minutes, and the mix is stirred an additional 15 minutes prior to addition of a solution of *(2R,3S,4S)*-5-

((4-methoxyphenyl)methoxy)-2,4-dimethyl-3-(*tert*-butyldimethylsilyloxy)-pentanal (18 mmol) in 50 mL of tetrahydrofuran. After 10 min, the mix is warmed to ambient temperature and stirred for 3 hours. Methanol (10 mL) is added, and the mixture is concentrated under vacuum and the residue filtered through a pad of silica gel using 1:1 ethyl acetate/hexanes. The filtrate is washed sequentially with sat. $Na_2S_2O_3$ and brine, dried over $MgSO_4$, filtered, and concentrated. The product is purified by silica gel chromatography.

EXAMPLE 78

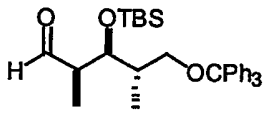
(2R,3R,4S,5R)-1-(triphenylmethoxy)-2,4-dimethyl-3-(*tert*-butyldimethylsilyloxy) oct-6-en-5-ol



A solution of *(2R,3S,4S,5R)*-2,4-dimethyl-3-(*tert*-butyldimethylsilyloxy)oct-6-en-1,5-diol (10 mmol) and triphenylmethyl chloride (11 mmol) in pyridine (25 mL) is stirred for 12 hours. The solvent is evaporated, and the product is purified by silica gel chromatography.

EXAMPLE 79

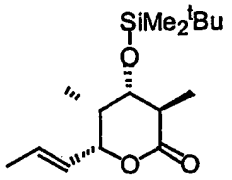
(2R,3S,4S)-5-(triphenylmethoxy)-2,4-dimethyl-3-(*tert*-butyldimethylsilyloxy)pentanal



A solution of *(2R,3R,4S,5R)*-1-(triphenylmethoxy)-2,4-dimethyl-3-(*tert*-butyldimethylsilyloxy)oct-6-en-5-ol (2.6 mmol) in a mix of 2,2-dimethylpropanol (30 mL), acetone (65 mL), and water (16 mL) is treated with osmium tetraoxide (0.08 mmol) and N-methylmorpholine N-oxide (13 mmol) for 11 hours at ambient temperature. The solution is diluted with 1 M NaH_2PO_4 and extracted with CH_2Cl_2. The extract is dried over Na_2SO_4, filtered, and evaporated to yield a crude triol intermediate. This material is dissolved in a mix of tetrahydrofuran (120 mL) and water (25 mL), and sodium metaperiodate (13 mmol) is added. After stirring vigorously for 24 hours, the mix is diluted with sat. $NaHCO_3$ and extracted with CH_2Cl_2. The extract is dried over Na_2SO_4, filtered, and evaporated. The product is purified by silica gel chromatography.

EXAMPLE 80

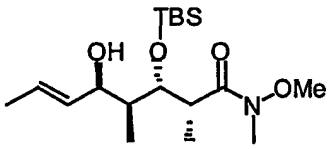
(2R,3S,4S,5R)-5-hydroxy-2,4-dimethyl-3-(*tert*-butyldimethylsilyloxy)-6-octenoic acid δ-lactone



A solution of *(2R,3S,4S,5R)*-3,5-dihydroxy-2,4-dimethyl-6-octenoic acid δ-lactone (10 mmol), 2,6-lutidine (15 mmol), and *tert*-butyldimethylsilyl triflate (11 mmol) in 10 mL of CH_2Cl_2 is stirred for 1 hour. The mixture is washed with water, then dried over $MgSO_4$, filtered, and evaporated. The product is purified by silica gel chromatography.

EXAMPLE 81

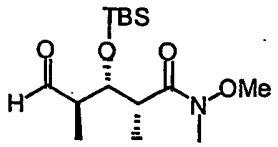
N-methoxy-N-methyl *(2R,3S,4S,5R)*-5-hydroxy-2,4-dimethyl-3-(*tert*-butyldimethylsilyloxy)-6-octenamide



A solution of 2 M trimethylaluminum in toluene (10 mmol) is added dropwise to a suspension of N,O-dimethylhydroxylamine hydrochloride (10 mmol) in 8 mL of CH_2Cl_2 at 0 °C. The resulting homogeneous solution is stirred for 30 min at ambient temperature. A solution of *(2R,3S,4S,5R)*-5-hydroxy-2,4-dimethyl-3-(*tert*-butyldimethylsilyloxy)-6-octenoic acid δ-lactone (2 mmol) in 4 mL of CH_2Cl_2 is added over 10 min, and the resulting solution is heated at reflux until complete consumption of starting material. Upon cooling, the mixture is poured into 1 M HCl, and extracted with CH_2Cl_2. The extract is washed sequentially with 5% $NaHCO_3$ and brine, dried over $MgSO_4$, filtered, and evaporated. The product is purified by silica gel chromatography.

EXAMPLE 82

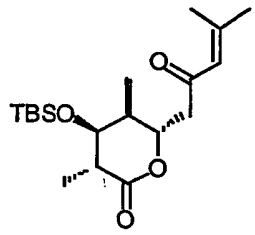
N-methoxy-N-methyl (*2R,3S,4R*)-2,4-dimethyl-3-(*tert*-butyldimethylsilyloxy)pentanamide



A solution of N-methoxy-N-methyl (*2R,3S,4S,5R*)-5-hydroxy-2,4-dimethyl-3-(*tert*-butyldimethylsilyloxy)-6-octenamide (2.6 mmol) in a mix of 2,2-dimethylpropanol (30 mL), acetone (65 mL), and water (16 mL) is treated with osmium tetraoxide (0.08 mmol) and N-methylmorpholine N-oxide (13 mmol) for 11 hours at ambient temperature. The solution is diluted with 1 M NaH_2PO_4 and extracted with CH_2Cl_2. The extract is dried over Na_2SO_4, filtered, and evaporated to yield a crude triol intermediate. This material is dissolved in a mix of tetrahydrofuran (120 mL) and water (25 mL), and sodium metaperiodate (13 mmol) is added. After stirring vigorously for 24 hours, the mix is diluted with sat. $NaHCO_3$ and extracted with CH_2Cl_2. The extract is dried over Na_2SO_4, filtered, and evaporated. The product is purified by silica gel chromatography.

EXAMPLE 83

(*2R,3S,4S,5R*)-5-hydroxy-7-oxo-2,4,9-trimethyl-3-(*tert*-butyldimethylsilyloxy)-8-decenoic acid δ-lactone

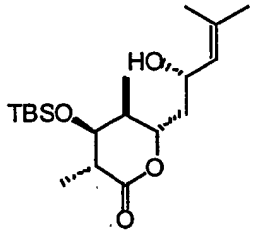


A 1.0 M solution of $TiCl_4$ in CH_2Cl_2 (10 mmol) is added dropwise to a –78 °C solution of N-methoxy-N-methyl (*2R,3S,4R*)-2,4-dimethyl-3-(*tert*-butyldimethylsilyloxy)-pentanamide (10 mmol) in 80 mL of CH_2Cl_2. To this mix is added 4-methyl-2-(trimethylsilyloxy)-1,3-pentadiene (20 mmol) and the mixture is stirred for 2 hours at –78 °C. The reaction is quenched by addition of 2:1 phosphate buffer (pH 8) and sat. $NaHCO_3$ (250 mL) and warmed to ambient temperature. The mix is extracted with CH_2Cl_2 and the extract is dried over $MgSO_4$, filtered, and evaporated. The residue is dissolved in 50 mL of 1:1 CH_2Cl_2/hexane and treated with trichloroacetic acid (15 mmol) on ice for 5 hours. The mix is diluted with hexane, washed sequentially with water, phosphate buffer (pH 8), and brine,

then dried with $MgSO_4$, filtered, and evaporated. The product is isolated by silica gel chromatography.

EXAMPLE 84

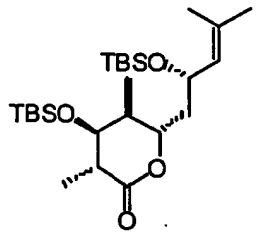
(2R,3S,4S,5R,7S)-5,7-dihydroxy-2,4,9-trimethyl-3-(*tert*-butyldimethylsilyloxy)-8-decenoic acid δ-lactone



A solution of *(2R,3S,4S,5R)*-5-hydroxy-7-oxo-2,4,9-trimethyl-3-(*tert*-butyldimethylsilyl-oxy)-8-decenoic acid δ-lactone (10 mmol) in toluene (250 mL) is cooled to –95 °C and treated with a 1.0 M solution of K-selectride (potassium tri-*sec*-butylborohydride) in tetrahydrofuran (12 mmol). After 2 hours, 0.5 mL of acetic acid is added, the solution is warmed to ambient temperature, and a mixture of phosphate buffer (pH 7) (325 mL) and 30% H_2O_2 (15 mL) is added. After stirring for 2 hours, the mix is extracted with CH_2Cl_2 and the extract is dried with $MgSO_4$, filtered, and concentrated. The product is isolated by silica gel chromatography.

EXAMPLE 85

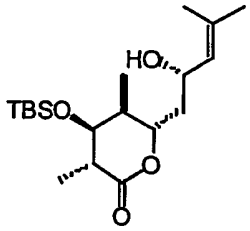
(2R,3S,4S,5R,7S)-5-hydroxy-2,4,9-trimethyl-3,7-di(*tert*-butyldimethylsilyloxy)-8-decenoic acid δ-lactone



A solution of *(2R,3S,4S,5R,7S)*-5,7-dihydroxy-2,4,9-trimethyl-3-(*tert*-butyldimethylsilyloxy)-8-decenoic acid δ-lactone (10 mmol), *tert*-butyldimethylsilyl chloride (20 mmol), and imidazole (30 mmol) in 50 mL of dimethylformamide is stirred at ambient temperature for 12 hours, then diluted with ether, washed with water, dried over $MgSO_4$, filtered, and concentrated. The product is isolated by silica gel chromatography.

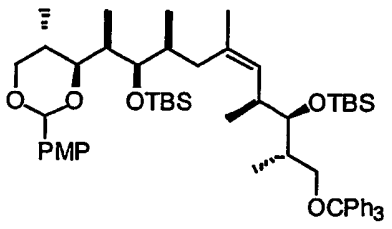
EXAMPLE 86

Alternate preparation of *(2R,3S,4S,5R,7S)*-5,7-dihydroxy-2,4,9-trimethyl-3-(*tert*-butyldimethylsilyloxy)-8-decenoic acid δ-lactone



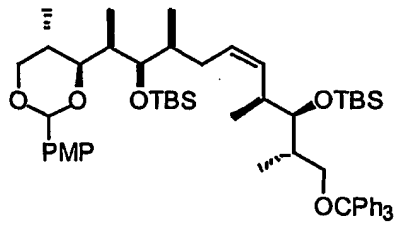
A 1.0 M solution of $TiCl_4$ in CH_2Cl_2 (10 mmol) is added dropwise to a –78 °C solution of N-methoxy-N-methyl (*2R,3S,4R*)-2,4-dimethyl-3-(*tert*-butyldimethylsilyloxy)-pentanamide (10 mmol) in 80 mL of CH_2Cl_2. To this mix is added 4-methyl-2-(trimethylsilyloxy)-1,3-pentadiene (20 mmol) and the mixture is stirred for 2 hours at –78 °C. The reaction is quenched by addition of 2:1 phosphate buffer (pH 8) and sat. $NaHCO_3$ (250 mL) and warmed to ambient temperature. The mix is extracted with CH_2Cl_2 and the extract is dried over $MgSO_4$, filtered, and evaporated. The residue is dissolved in 50 mL of acetonitrile and added to a –40 °C solution of tetramethylammonium triacetoxyborohydride (80 mmol) and acetic acid (44 mL) in 44 mL of acetonitrile which had been allowed to stir for 30 minutes at ambient temperature prior to cooling. The reaction is allowed to proceed for 18 hours at –40 °C, then is quenched by addition of 0.5 M aqueous sodium potassium tartrate and warmed to ambient temperature. The mix is extracted with CH_2Cl_2 and the extract is dried over $MgSO_4$, filtered, and evaporated. The residue is dissolved in 50 mL of 1:1 CH_2Cl_2/hexane and treated with trichloroacetic acid (15 mmol) on ice for 5 hours. The mix is diluted with hexane, washed sequentially with water, phosphate buffer (pH 8), and brine, then dried with $MgSO_4$, filtered, and evaporated. The product is isolated by silica gel chromatography.

EXAMPLE 87



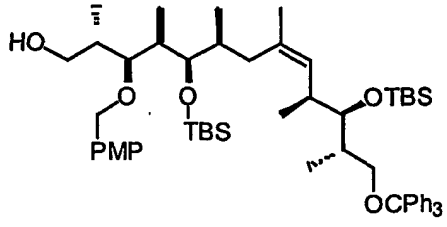
A 1 M solution of sodium hexamethyldisilazide in tetrahydrofuran (10 mmol) is added to a suspension of (*4S,5R,6R,7S*)-2-triphenylphosphonium-4,6,8-trimethyl-7,9-dihydroxy-5-(^{t}butyldimethylsilyloxy)nonan-2-ol 7,9-(4-methoxybenzylidene)acetal iodide (10 mmol) in 10 mL of tetrahydrofuran. After 15 minutes, a solution of (*2R,3S,4S*)-5-(triphenylmethoxy)-2,4-dimethyl-3-(*tert*-butyldimethylsilyloxy)pentanal (10 mmol) is added and the mix is stirred for 3 hours. The mixture is concentrated under vacuum and the residue filtered through a pad of silica gel using 1:1 ethyl acetate/hexanes. The filtrate is concentrated. The product is purified by silica gel chromatography.

EXAMPLE 88



A 1 M solution of sodium hexamethyldisilazide in tetrahydrofuran (10 mmol) is added to a suspension of (*3S,4R,5R,6S*)-1-triphenylphosphonium-3,5,7-trimethyl-6,8-dihydroxy-4-(^{t}butyldimethylsilyloxy)octane 6,8-(4-methoxybenzylidene)acetal iodide (10 mmol) in 10 mL of tetrahydrofuran. After 15 minutes, a solution of (*2R,3S,4S*)-5-(triphenylmethoxy)-2,4-dimethyl-3-(*tert*-butyldimethylsilyloxy)pentanal (10 mmol) is added and the mix is stirred for 3 hours. The mixture is concentrated under vacuum and the residue filtered through a pad of silica gel using 1:1 ethyl acetate/hexanes. The filtrate is concentrated. The product is purified by silica gel chromatography.

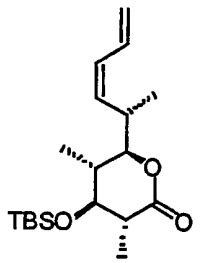
EXAMPLE 89



A 1M solution of diisobutylaluminum hydride in toluene (30 mmol) is added to a 0 °C solution of the product of Example 49 (10 mmol) in 100 mL of CH_2Cl_2, and the mixture is stirred for 5 hours. Aqueous phosphate buffer (pH 7.0) is added dropwise to quench, the mixture is diluted with 100 mL of CH_2Cl_2, poured into 100 mL of saturated sodium

potassium tartrate, and extracted with CH_2Cl_2. The extract is dried over $MgSO_4$, filtered, and evaporated. The crude product is purified by silica gel chromatography.

EXAMPLE 90



A solution of 4-methoxybenzyl alcohol (11 mmol) in 10 mL of tetrahydrofuran is added dropwise to a suspension of sodium hydride (12 mmol) in 50 mL of tetrahydrofuran. After cessation of gas evolution, the mixture is treated with a solution of (*7Z*)-(*2R,3S,4R,5S,6S*)-5-hydroxy-3-(*t*butyldimethylsilyloxy)-2,4,6-trimethyldeca-7,9-dienoate δ-lactone (10 mmol) in 10 mL of tetrahydrofuran. The reaction is monitored by thin-layer chromatography. Upon disappearance of the lactone, the mixture is cooled to 0 °C and treated with trifluoromethanesulfonic anhydride (12 mmol). After formation of the triflate, the mixture is treated with 2,3-dichloro-1,5-dicyano-1,4-benzoquinone (12 mmol) and water for 10 minutes at 0 °C, then warmed to ambient temperature prior to addition of triethylamine (12 mmol). The reaction is monitored by thin-layer chromatography. When complete, the mixture is quenched by addition of sat. aq. $NaHCO_3$ and extracted with ether. The extract is washed with brine, dried over $MgSO_4$, filtered, and evaporated. The product is purified by silica gel chromatography.

The compounds of the present invention generally include a plurality of chiral centers and optionally a double bond. Although preferred embodiments (preferred isomers) are used to illustrate the invention, the present invention encompasses all stereo and geometric isomers. All scientific and patent publications referenced herein are hereby incorporated by reference. The invention having now been described by way of written description and example, those of skill in the art will recognize that the invention can be practiced in a variety of embodiments, that the foregoing description and example is for purposes of illustration and not limitation of the following claims.

What is claimed is:

1. A method for making a naturally-occurring polyketide comprising

fermenting a host cell containing an expression vector,

said vector comprising a recombinant gene encoding a polyketide synthase,

said synthase comprising modules from at least two different naturally-occurring polyketide synthases, wherein at least one of the naturally-occurring polyketide synthases does not naturally produce said polyketide, and

optionally isolating said polyketide from the fermentation medium.

2. The method of Claim 1, wherein the polyketide is an epothilone analog or a discodermolide analog.

3. A method for making a first compound useful in synthesizing a second compound, wherein

said second compound contains four or more chiral centers, and
said first compound contains two or more chiral centers,
said method comprising expressing in a recombinant host cell a recombinant, non-naturally occurring polyketide synthase that produces said first compound.

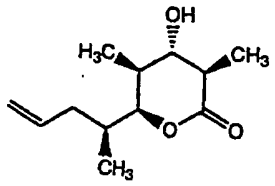
4. The method of Claim 3, wherein the first compound contains at least 3 chiral centers, and the second compound contains at least 5 chiral centers.

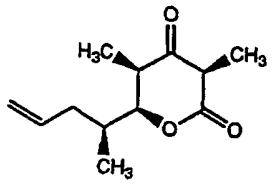
5. The method of Claim 3, wherein the second compound contains at least 10 chiral centers.

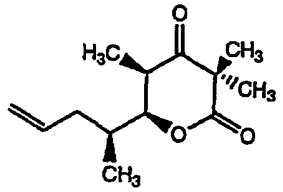
6. The method of Claim 3, wherein the recombinant, non-naturally occurring PKS is either a portion of a naturally occurring PKS gene or is composed of portions of two or more naturally occurring PKS genes.

7. The method of Claim 3, wherein the second compound is an epothilone analog or a discodermolide analog.

8. The method of Claim 3, wherein said first compound is selected from the group consisting of:



,


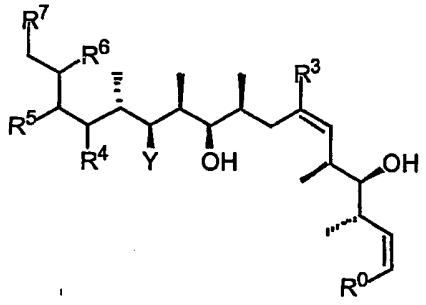


, and

9. The method of Claim 3, wherein said first compound is selected from the group consisting of 14-chloro-14-desmethyl-6-deoxyerythronolide B and 14-desmethyl-6-deoxy-14-(phenylthio)erythronolide B.

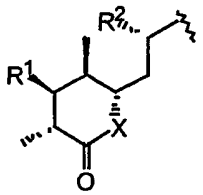
10. The method of Claim 3, wherein said first compound is selected from the group consisting of:14-chloro-14-desmethyl-6-deoxy-8-hydroxyerythronolide B and 14-desmethyl-6-deoxy-8-hydroxy-14-(phenylthio)erythronolide B.

11. A compound of the formula:



wherein

R^0 is C1-C8 alkyl, C1-C8 alkenyl, C1-C8 alkynyl, aryl, 2-phenylethyl, 2-(3-hydroxyphenyl)ethyl, or a group of the formula



wherein R^1 and R^2 are each independently hydrogen, hydroxyl, or a hydroxyl protecting group; and X is O, NH, or N-alkyl;

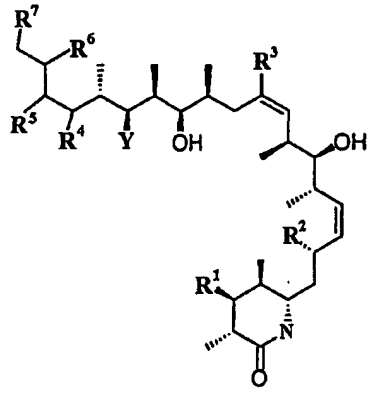
R^3 is hydrogen, C_1-C_{10} alkyl or aryl;

R^4, R^5, R^6, and R^7 are each hydrogen, or R^4 and R^5 together form a double bond and R^6 and R^7 together form a double bond; and

Y is hydroxyl, amino, -OC(=O)NH_2 or -NHC(=O)NH_2,

with the proviso that when R^3 is hydrogen or C_1-C_6 alkyl that: (i) at least one of R^1 and R^2 is not hydroxyl, or (ii) R^4, R^5, R^6, and R^7 are each hydrogen, or (iii) X is nitrogen, or (iv) Y is hydroxyl, amino, or -NHC(=O)NH_2, or (v) any combination of (i) through (iv).

12. The compound of Claim 11 that is:



wherein

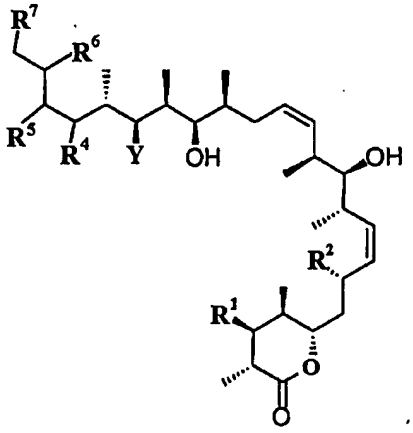
R^1 and R^2 are each independently hydrogen, hydroxyl, or a hydroxyl protecting group;

R^3 is hydrogen, C_1-C_{10} alkyl or aryl;

R^4, R^5, R^6, and R^7 are each hydrogen, or R^4 and R^5 together form a double bond and R^6 and R^7 together form a double bond; and,

Y is hydroxyl, amino, -OC(=O)NH_2 or -NHC(=O)NH_2.

13. The compound of Claim 11 that is:



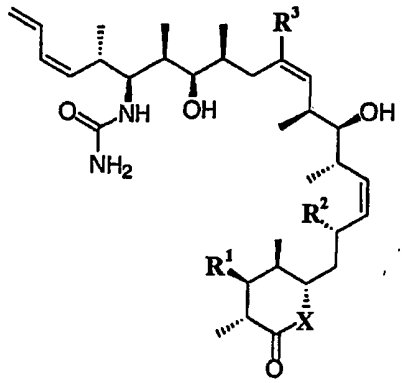
wherein

R^1 and R^2 are each independently hydrogen, hydroxyl, or a hydroxyl protecting group;

R^4, R^5, R^6, and R^7 are each hydrogen, or R^4 and R^5 together form a double bond and R^6 and R^7 together form a double bond; and,

Y is hydroxyl, amino, $-OC(=O)NH_2$ or $-NHC(=O)NH_2$, provided at least one of R^1 and R^2 is not hydroxyl.

14. The compound of Claim 11 that is:



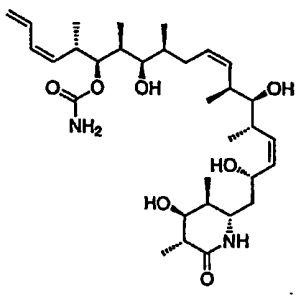
wherein

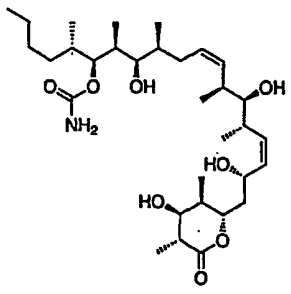
R^1 and R^2 are each independently hydrogen, hydroxyl, or a hydroxyl protecting group;

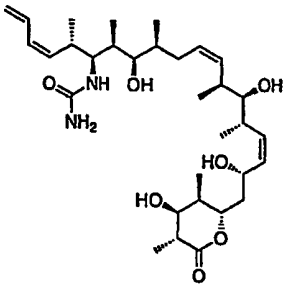
R^3 is hydrogen, C_1-C_{10} alkyl or aryl; and,

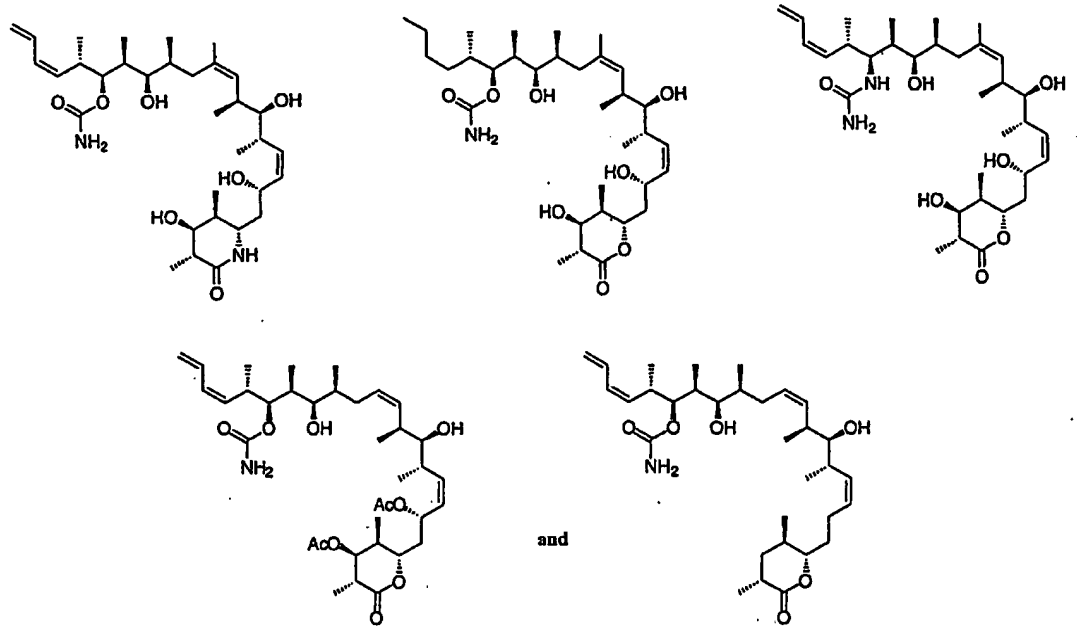
X is oxygen or nitrogen.

15. The compounds of Claim 11 that are selected from the group consisting of:



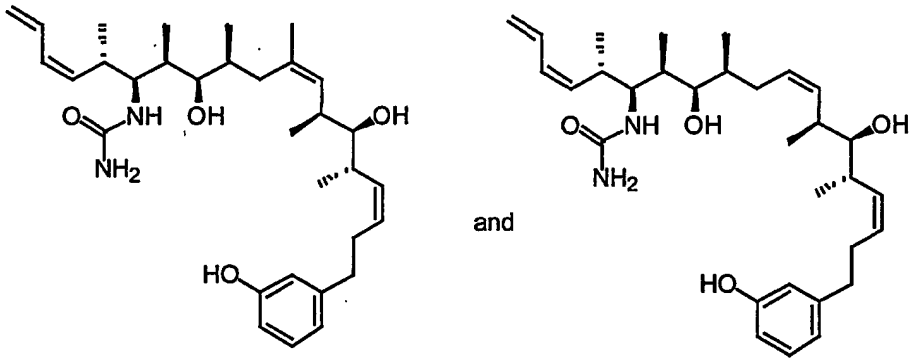






and

16. The compounds of Claim 11 that are selected from the group consisting of:



and

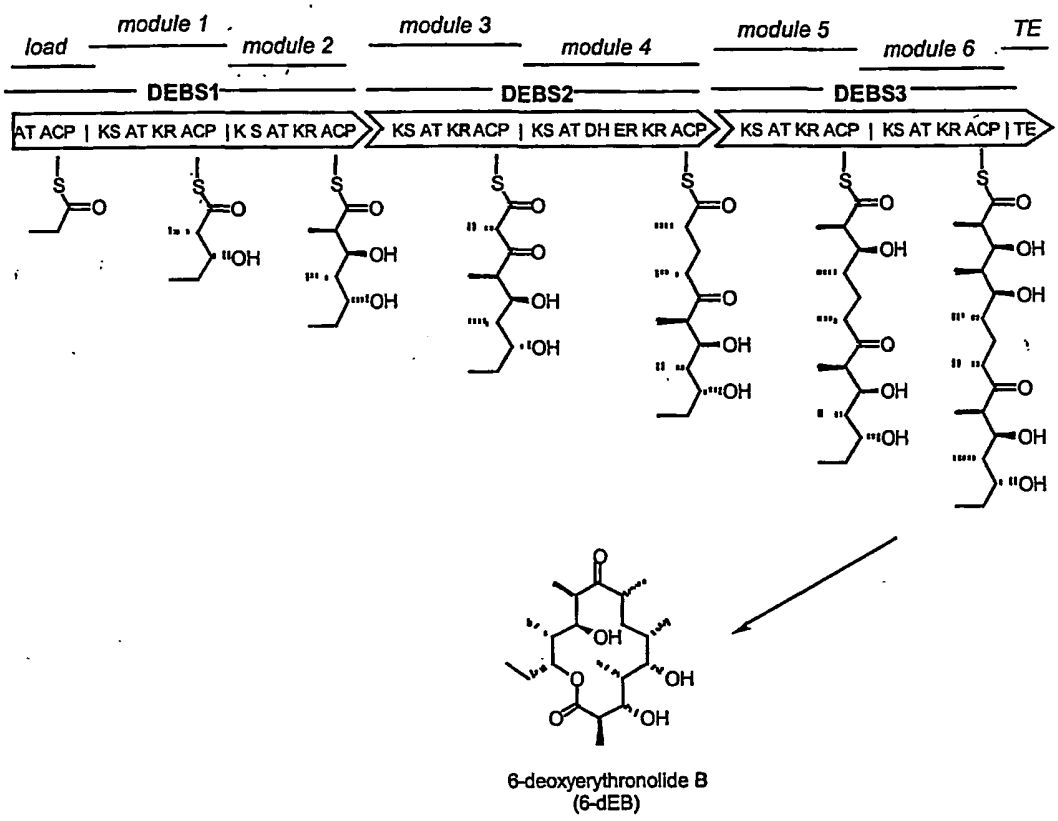

load
module 1
module 2
module 3
module 4
module 5
module 6
TE
DEBS1
DEBS2
DEBS3
AT ACP | KS AT KR ACP | K S AT KR ACP
KS AT KR ACP | KS AT DH ER KR ACP
KS AT KR ACP | KS AT KR ACP | TE
6-deoxyerythronolide B
(6-dEB)

FIGURE 1

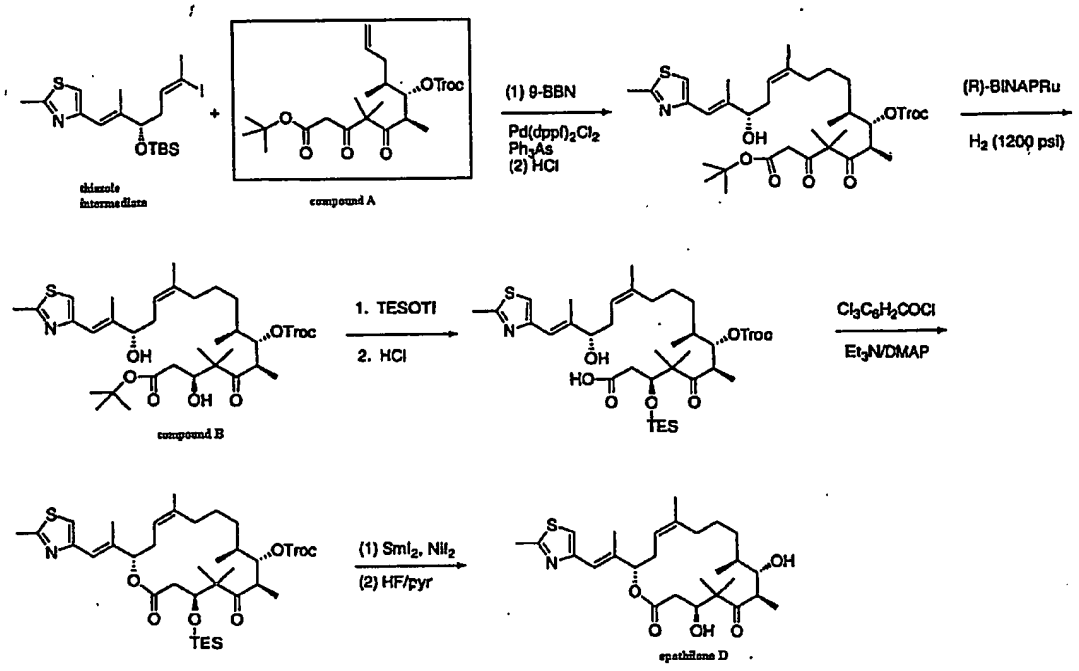

OTBS
OTroc
(1) 9-BBN
Pd(dppf)2Cl2
Ph3As
(2) HCl
(R)-BINAPRu
H2 (1200 psi)
1. TESOTf
2. HCl
Cl3C6H2COCl
Et3N/DMAP
TES
(1) SmI2, NiI2
(2) HF/pyr
OH

FIGURE 2

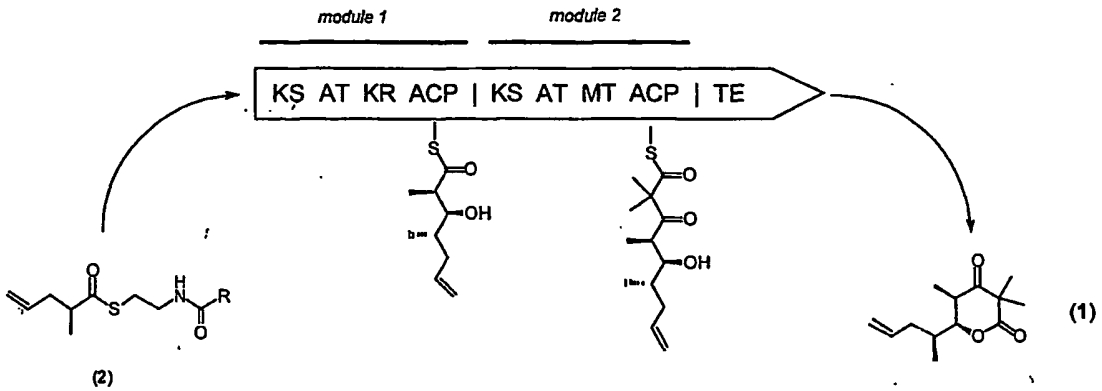


FIGURE 3

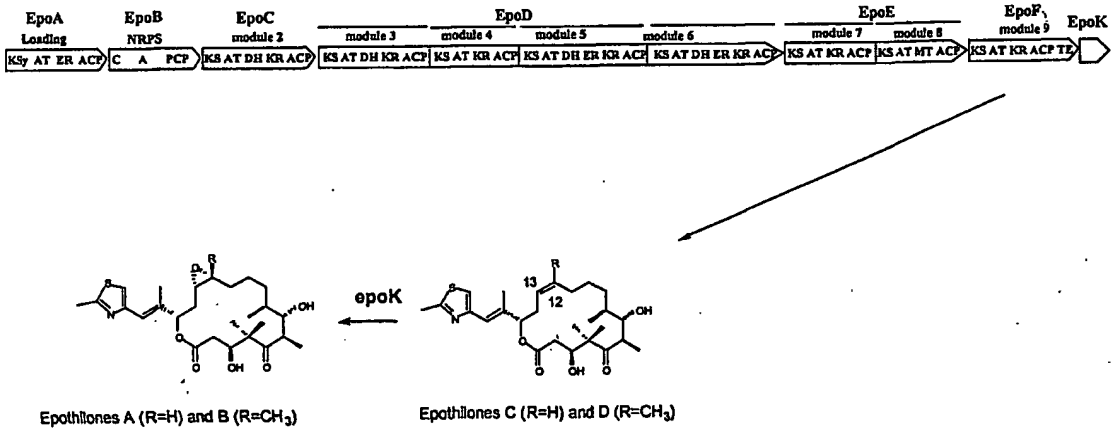

EpoA
Loading
KSy AT ER ACP
EpoB
NRPS
C A PCP
EpoC
module 2
KS AT DH KR ACP
EpoD
module 3
KS AT DH KR ACP
module 4
KS AT KR ACP
module 5
KS AT DH ER KR ACP
module 6
KS AT DH ER KR ACP
EpoE
module 7
KS AT KR ACP
module 8
KS AT MT ACP
EpoF
module 9
KS AT KR ACP TE
EpoK
epoK
Epothilones A (R=H) and B (R=CH3)
Epothilones C (R=H) and D (R=CH3)

FIGURE 4

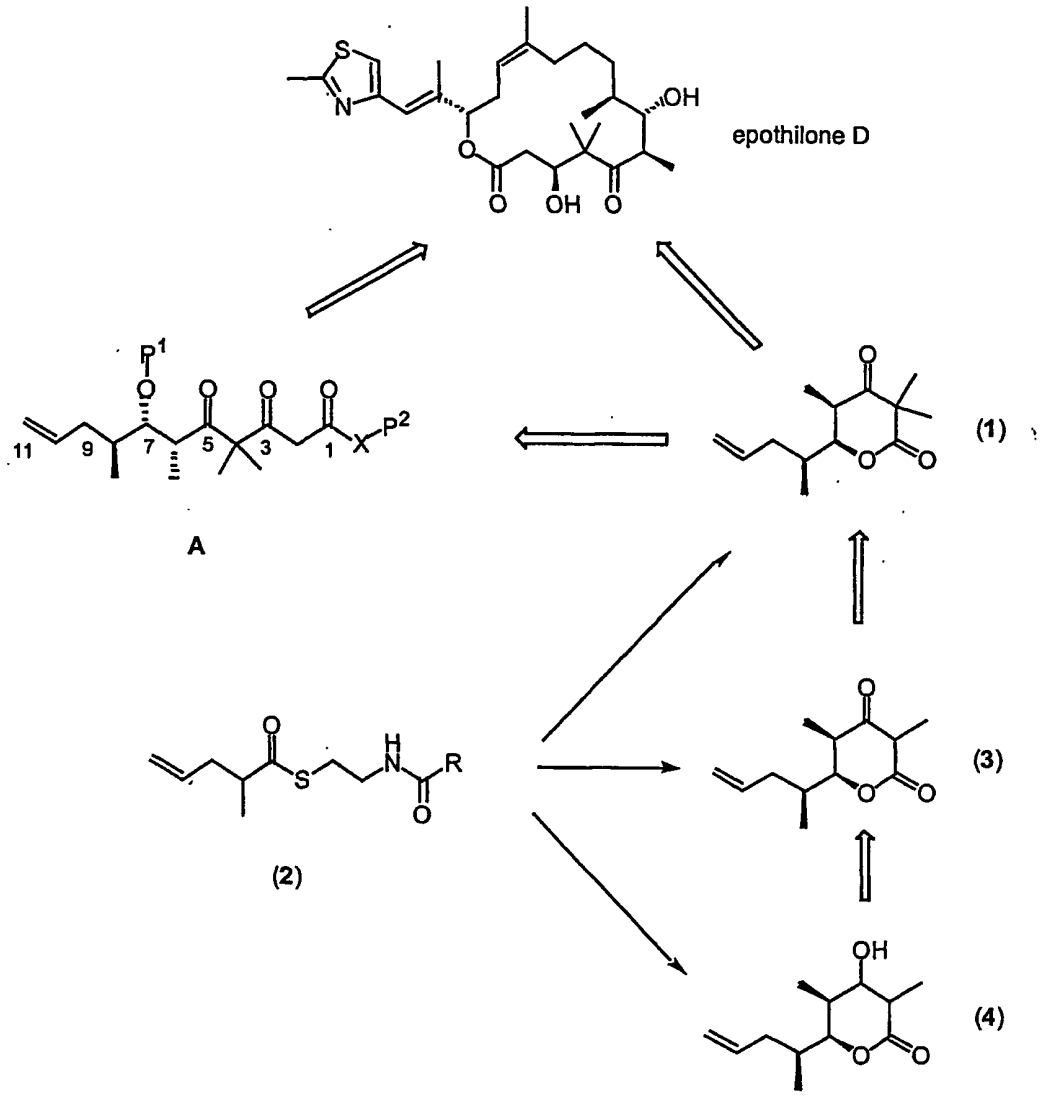


FIGURE 5

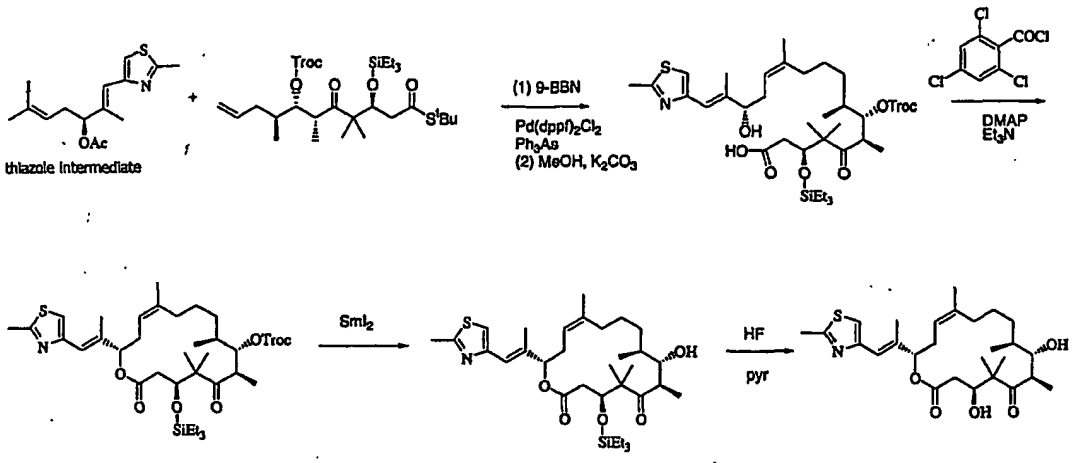

thiazole intermediate
(1) 9-BBN
Pd(dppf)2Cl2
Ph3As
(2) MeOH, K2CO3
DMAP
Et3N
SmI2
HF
pyr

FIGURE 6

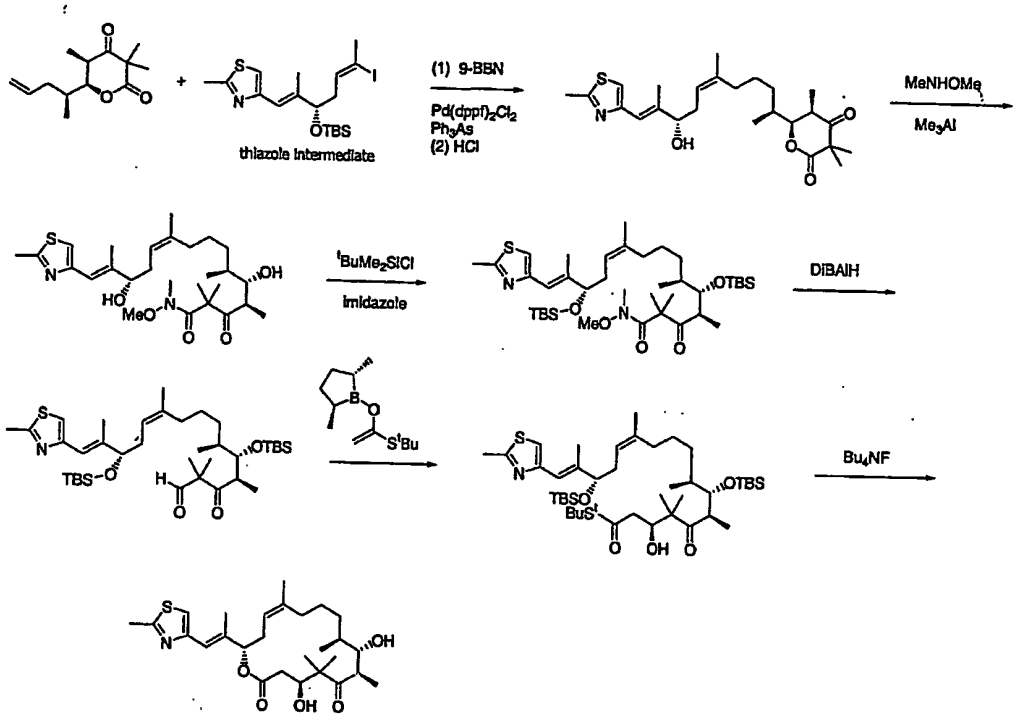

(1) 9-BBN
Pd(dppf)2Cl2
Ph3As
(2) HCl
thiazole intermediate
MeNHOMe
Me3Al
tBuMe2SiCl
Imidazole
DIBAlH
Bu4NF

FIGURE 7

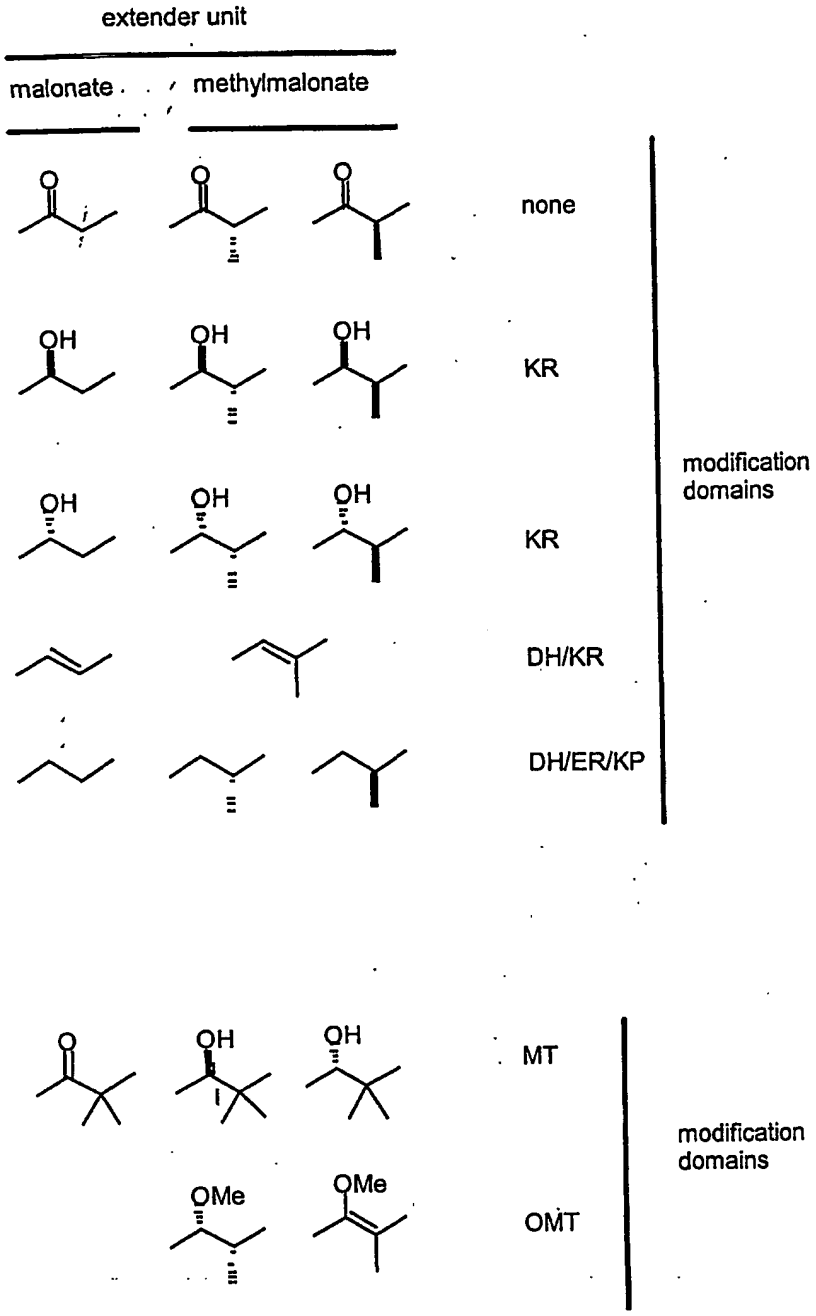

extender unit
malonate
methylmalonate
none
KR
KR
DH/KR
DH/ER/KP
modification domains
MT
OMT
modification domains

FIGURE 8

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

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(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

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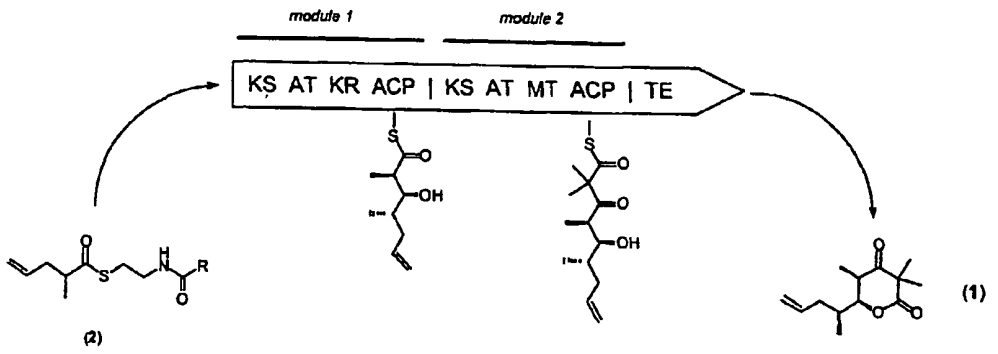
Published:
— *with international search report*

(88) Date of publication of the international search report:
6 September 2002

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: BIO-INTERMEDIATES FOR USE IN THE CHEMICAL SYNTHESIS OF POLYKETIDES



(57) Abstract: The present invention relates to compounds made by a subset of modules from one or more polyketide synthase ("PKS") genes that are used as starting material in the chemical synthesis of novel molecules, particularly naturally occurring polyketides or derivatives thereof. The biologically derived intermediates ("bio-intermediates") generally represent particularly difficult compounds to synthesize using traditional chemical approaches due to one or more stereocenters. In one aspect of the invention, an intermediate in the synthesis of epothilone is provided that feeds into the synthetic protocol of Danishefsky and co-workers. In another aspect of the invention, intermediates in the synthesis of discodermolide are provided that feed into the synthetic protocol of Smith and co-workers. By taking advantage of the inherent stereochemical specifity of biological processes, the syntheses of key intermediates and thus the overall syntheses of compounds like epothilone and discodermolide are greatly simplified.

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INTERNATIONAL SEARCH REPORT

International application No
PCT/US 01/25112

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12P17/06 C12P17/08 C12P17/12 C12P17/16 C12P17/18
C12P13/02 C12P7/26 C12P11/00 C07D309/30 C07D211/76
C07C275/24

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12P C07D C07C

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data, PAJ, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 00 31247 A (KOSAN BIOSCIENCES INC) 2 June 2000 (2000-06-02) cited in the application the whole document page 42, line 4 -page 46, line 5 page 57, line 19-33 examples 6,8 ---	1-7
X	GOKHALE R S ET AL.: "Dissecting and exploiting intermodular communication in polyketide synthases" SCIENCE, vol. 284, 16 April 1999 (1999-04-16), pages 482-485, XP002155932 ISSN: 0036-8075 the whole document --- -/--	1,3,4,6

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
16 January 2002	25. 04 2002

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	van de Kamp, M

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 01/25112

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	RANGANATHAN A ET AL.: "Knowledge-based design of bimodular and trimodular polyketide synthases based on domain and module swaps: a route to simple statin analogues" CHEMISTRY AND BIOLOGY, vol. 6, no. 10, October 1999 (1999-10), pages 731-741, XP000879061 ISSN: 1074-5521 the whole document	1,3,4,6
X	WO 00 44717 A (KOSAN BIOSCIENCES INC ;ASHLEY GARY (US); BURLINGAME MARK ALMA (US)) 3 August 2000 (2000-08-03) page 9, line 31 -page 13, line 2 page 18, line 6 -page 22, line 15 examples 15-18,24 claims 5,14,18	3-6
X	WO 99 03986 A (KOSAN BIOSCIENCES INC) 28 January 1999 (1999-01-28) examples 1-4,6 claims 6,9	3-6
X	WO 00 24907 A (KOSAN BIOSCIENCES INC) 4 May 2000 (2000-05-04) cited in the application the whole document	3-6
A	NICOLAOU K C ET AL.: "Chemical Biology of Epothilones" ANGEWANDTE CHEMIE (INTERNATIONAL EDITION), vol. 37, no. 15, August 1998 (1998-08), pages 2014-2045, XP002131418 ISSN: 0570-0833 cited in the application the whole document	2,7
A	WO 00 04865 A (UNIV PENNSYLVANIA) 3 February 2000 (2000-02-03) the whole document	

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 01/25112

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1-7 (partially); 8 (complete)

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: 1-7 (all partially); 8 (completely)

A method for making a naturally occurring polyketide, e.g., an epothilone analog, comprising, fermenting a host cell containing an expression vector, said vector comprising a recombinant gene encoding a polyketide synthase (PKS), said PKS comprising modules from at least two different naturally occurring PKSs wherein at least one of these PKSs does not naturally produce said polyketide, and optionally isolating said polyketide.
A method for making a first compound useful in synthesizing a second compound, said second compound containing four or more or five or more or ten or more chiral centers (e.g., an epothilone analog), said first compound containing two or more or three or more chiral centers (e.g., one of the nonenoate delta-lactones of claim 8), said method comprising expressing in a recombinant host cell a recombinant non-naturally occurring PKS (e.g., either a portion of a naturally occurring PKS or a PKS composed of portions of two or more naturally occurring PKSs) that produces said first compound.

2. Claims: 1-7 (all partially); 9, 10 (completely)

A method for making a naturally occurring polyketide, e.g., a discodermolide analog, comprising, fermenting a host cell containing an expression vector, said vector comprising a recombinant gene encoding a polyketide synthase (PKS), said PKS comprising modules from at least two different naturally occurring PKSs wherein at least one of these PKSs does not naturally produce said polyketide, and optionally isolating said polyketide.
A method for making a first compound useful in synthesizing a second compound, said second compound containing four or more or five or more or ten or more chiral centers (e.g., a discodermolide analog), said first compound containing two or more or three or more chiral centers (e.g., one of the 6-deoxyerythronolides of claims 9 and 10), said method comprising expressing in a recombinant host cell a recombinant non-naturally occurring PKS (e.g., either a portion of a naturally occurring PKS or a PKS composed of portions of two or more naturally occurring PKSs) that produces said first compound.

3. Claims: 11-16

Compounds according to claims 11-16.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/US 01/25112

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 0031247 A	02-06-2000	AU 1737700 A	13-06-2000
		CN 1333820 T	30-01-2002
		EP 1135470 A2	26-09-2001
		WO 0031247 A2	02-06-2000
		US 6303342 B1	16-10-2001
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		EP 1144375 A2	17-10-2001
		WO 0044717 A2	03-08-2000
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		EP 1124968 A2	22-08-2001
		WO 0024907 A2	04-05-2000
WO 0004865 A	03-02-2000	US 6096904 A	01-08-2000
		AU 5219099 A	14-02-2000
		EP 1105383 A2	13-06-2001
		WO 0004865 A2	03-02-2000
		US 6242616 B1	05-06-2001

Kosan Biosciences, Inc.

TO: Peter Licari

FROM: Janice Lau

DATE: 4/29/02

SUBJECT: Ansamitocin Strain History

1. *Actinosynnema pretiosum* N-1231 (ATCC 31565) is a high-producing mutant derived from *A. pretiosum* N-1001 by treatment with ethidium bromide [1]. The parent strain N-1001 was isolated from a grass blade collected in Shiga Prefecture, Japan [2].

2. The *A. pretiosum* strain N-1231 was received by Chris Reeves on 5-30-2001 from ATCC and was streaked on ISP2 agar plates for clonal isolation (Notebook K131-141). Three isolates A, B, and C were selected and grown in TSB-GP medium (30 g/L tryptic soy broth, 10 g/L glucose, and 25 mM PIPES, pH 6.8) for cell bank preparation. The resulting mycelial cell banks are designated as Pre Master Cell Banks (PCB) and are stored at –80°C at Kosan.

3. Clones A, B, and C were streaked on ISP2 agar plates and assessed for clonal variability. Three isolates of each clone (A1-A3, B5-B7, and C8-C10) were evaluated and were found to produce 148 ± 20 mg/L of ansamitocin P-3 (Notebook K202-108).

4. Clone C10 was grown on ISP2 agar plates containing 5 g/L calcium chloride dihydrate. The resulting spores were used for the preparation of the Master Cell Bank (MCB) on 8-20-2001 (Notebook K202-94), which is stored at -80°C at Kosan.

5. One vial of spores from the MCB was grown on ISP2 agar plates containing 5 g/L calcium chloride dihydrate. The resulting spores were used for the preparation of the Working Cell Banks (WCB) on 9-25-2001 and 9-27-2001 (Notebook K131-183). The cell banks are stored at -80°C at Kosan.

References

1. Tanida S, Izawa M, Hasegawa T. 1981. Ansamitocin analogs from a mutant strain of *Nocardia*. I. Isolation of the mutant, fermentation and antimicrobial properties. J Antibiotics (Tokyo) 34:489-495.

2. Tanida S, Hasegawa T, Muroi M, Higashide E. 1980. Dnacins, new antibiotics. I. Producing organism, fermentation, and antimicrobial activities. J Antibiotics (Tokyo) 33:1443-1448.

Mike Sherrill

From: Gillian Cropp
Sent: Wednesday, February 18, 2004 11:02 AM
To: Helen Street
Cc: Sherry Slaughter; Mike Sherrill
Subject: RE: KOS-152 - PK Manual & Labels

Helen,

Traci is RIGHT and I am very very cross. The synopsis in the new version of the protocol is correct (P6). The table in the schedule (p27/28) has been changed, probably when the 48 hour sample at MTD was added.
It is now wrong.
I checked the original protocol and the table correctly listed the PK sample for KOS-862 as 25.5 hours post, the new version lists it as 24.5 hours. I do not know who changed this number when the 48 hour sample was added.
What to do? The vital blood draw is the exactly 24 hour post for carboplatin. If the incorrect number means the patients get two blood sticks, I suppose we have to live with it.
At the SIV the emphasis must be on the exactly 24 hour post infusion for carboplatin.

Yours, very cranky, Gillian
-----Original Message-----
From: Mike Sherrill
Sent: Tuesday, February 17, 2004 4:28 PM
To: Helen Street; Gillian Cropp
Cc: Sherry Slaughter
Subject: FW: KOS-152 - PK Manual & Labels

Can you please have a look and provide comments back to Traci.

Thanks,
Mike

-----Original Message-----
From: THASTINGS@prevalere.com [mailto:THASTINGS@prevalere.com]
Sent: Tuesday, February 17, 2004 2:16 PM
To: Mike Sherrill
Subject: KOS-152 - PK Manual & Labels

Hi Mike-

Attached please find [4] files for your review. These files make up the manual we would send to the clinical sites. Once these documents are printed we bind them with tab dividers (e.g. Processing Instructions, Shipping Instructions, Labels, Transaction Reports).

I have printed an example of the labels for one patient and will send them to you via Federal Express tomorrow for your review. You should receive them on Thursday.

I did have one question/comment on the timepoint for the PK draws. I noticed on page 28 of the manual there is a timepoint of 25.5 hours and on page 6 the timepoint is 24.5 hours. Should this timepoint be 24.5 or 25.5? I put 25.5 hours on the transaction reports and the tube labels, but can easily change this if it needs to be 24.5 hours.

Please feel free to contact me at your convenience with your comments.

The PK kits are assembled and ready to go. Once we receive your comments on the manual and labels, the only steps left for us will be to generate and print all the labels along with a manual for each site.
<<InstructionBookletCover.doc>> <<InstructionsProcessing.doc>>

<<InstructionsShipping.doc>> <<TransactionReport.xls>>

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